

Fig. 1

Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined.

<i>Bet v 1</i> sense	5'- AATTATGAGACTGAGACC <u>A</u> CCTCTGTTATCCCAGCAGCTCG	-3'
<i>Bet v 1</i> non-sense	3'- TTAATACTCTGACTCTGG <u>T</u> GGAGACAATAGGGTCGTCGAGC	-5'
sense primer	5'- TGAGACC <u>C</u> CCTCTGTTATCCCAG	-3'
non-sense primer	3'- ATACTCTGACTCTGG <u>G</u> GGAGACA	-5'

Fig. 2

Oligonucleotide primers for site directed mutagenesis of  
Bet v 1 (No. 2801).

all	sense	1: 183Bv, 15-mer 5'-GTTGCCAACGATCAG
1	sense	2: 184Bv, 23-mer 5'-TGAGACCCCTCTGTTATCCCAG
1	non-sense	3: 185Bv, 23-mer 5'-ACAGAGGGGTCTCAGTCTCATA
2	sense	4: 186Bv, 31-mer 5'-GATACCCCTCTTCCACAGGTTGCACCCCAAG
2	non-sense	5: 187Bv, 31-mer 5'-ACCTGTGAAAGAGGGTATCGCCATCAAGGA
3	sense	6: 188Bv, 23-mer 5'-AACATTTCAGGAAATGGAGGGCC
3	non-sense	7: 189Bv, 23-mer 5'-TTTCCTGAAATGTTTCAACACT
4	sense	8: 190Bv, 23-mer 5'-TTAAGAACATCAGCTTTCCCGAA
4	non-sense	9: 191Bv, 23-mer 5'-AGCTGATGTTCTTAATGGTTCCA
5	sense	10: 192Bv, 23-mer 5'-GGACCATGCAACTTCAAATACA
5	non-sense	11: 193Bv, 23-mer 5'-AGTTTGCATGGTCCACCTCATCA
6	sense	12: 194Bv, 23-mer 5'-TTTCCTCAGGCCTCCCTTTCAA
6	non-sense	13: 195Bv, 23-mer 5'-AGGCCTGAGGGAAAGCTGATCTT
7	sense	14: 196Bv, 24-mer 5'-TGAAGGATCTGGAGGGCCTGGAAC
7	non-sense	15: 197Bv, 24-mer 5'-CCCTCCAGATCCTTCAATGTTTTTC
8	sense	16: 198Bv, 24-mer 5'-GGCAACTGGTGATGGAGGATCCAT
8	non-sense	17: 199Bv, 24-mer 5'-CCATCACCAGTTGCCACTATCTTT
all	non-sense	18: 200Bv, 15-mer 5'-CATGCCATCCGTAAG

Fig. 3

## Overview of all Bet v 1 mutations

1 (A-C)	
GGTGTGTTTAATTATGAGACTGAGACCACTCTGTTATCCCAGCAGCTCGACTGTTCAAG	60
G V F N Y E T E T T-P S V I P A A R L F K	20
9 (A-G) 2 (A-C) 2 (A-C)	
GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT	120
A F I L D-G G D N-T L F P K-Q V A P Q A I S S	40
3 (GA-TC) 7 (AA-TC) 4 (G-C) 6 (GA-TC)	
GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAGAAGATCAGCTTTCCCGAA	180
V E N I E-S G N-S G G P G T I K K-N I S F P E-S	60
5 (CA-TG)	
GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCAACAAACTTCAAA	240
G L P F K Y V K D R V D E V D H T-A N F K	80
TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC	300
Y N Y S V I E G G P I G D T L E K I S N	100
10 (GAG-CAC) 8 (CCC-TGG)	
GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC	360
E I K I V A T P-G D G G S I L K I S N K Y	120
CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC	420
H T K G D H E V K A E Q V K A S K E M G	140
GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAACATA	480
E T L L R A V E S Y L L A H S D A Y N stop	159

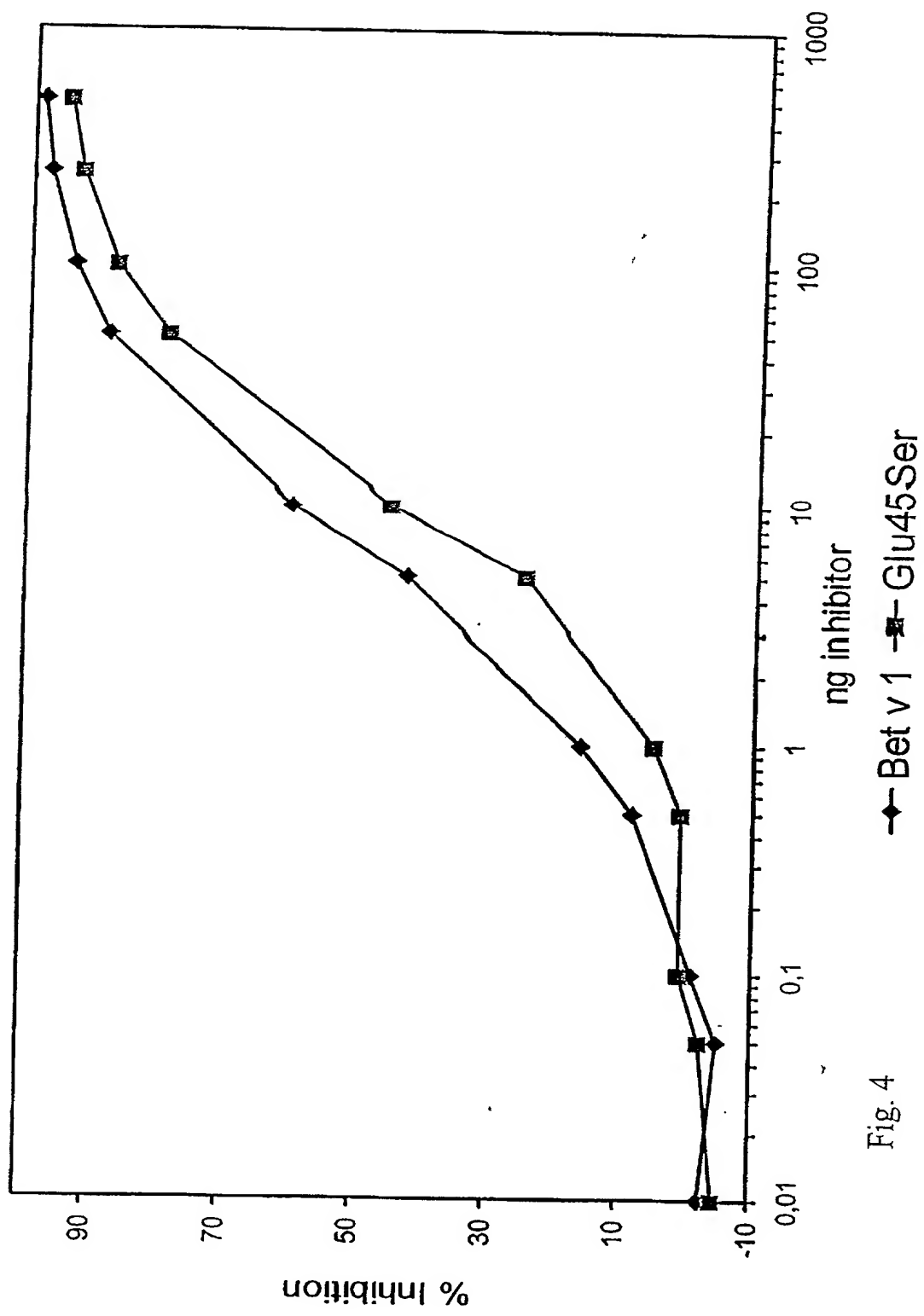


Fig. 4

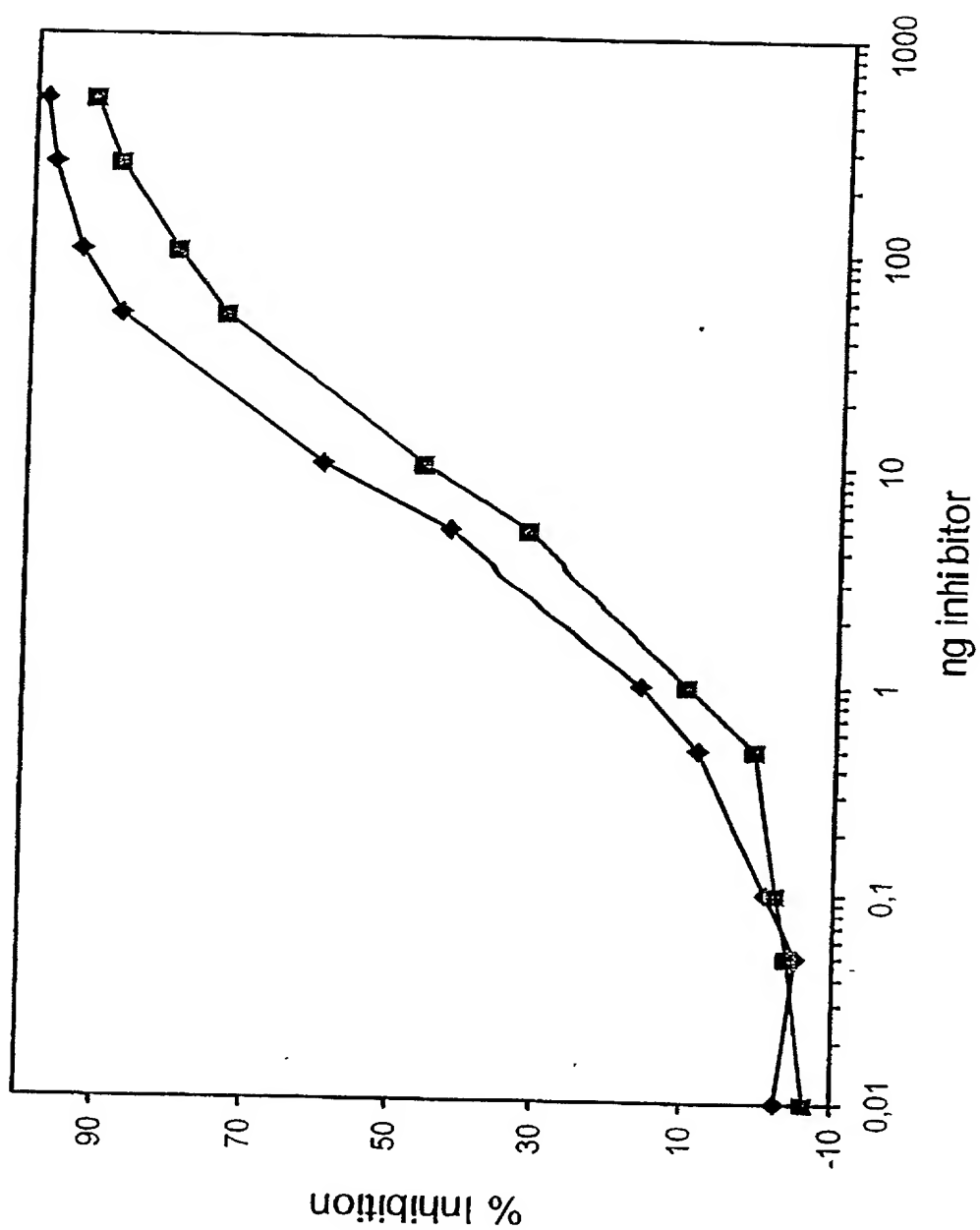


Fig. 5 —◆— Bet v 1 —■— Asn28Thr+Lys32Gln

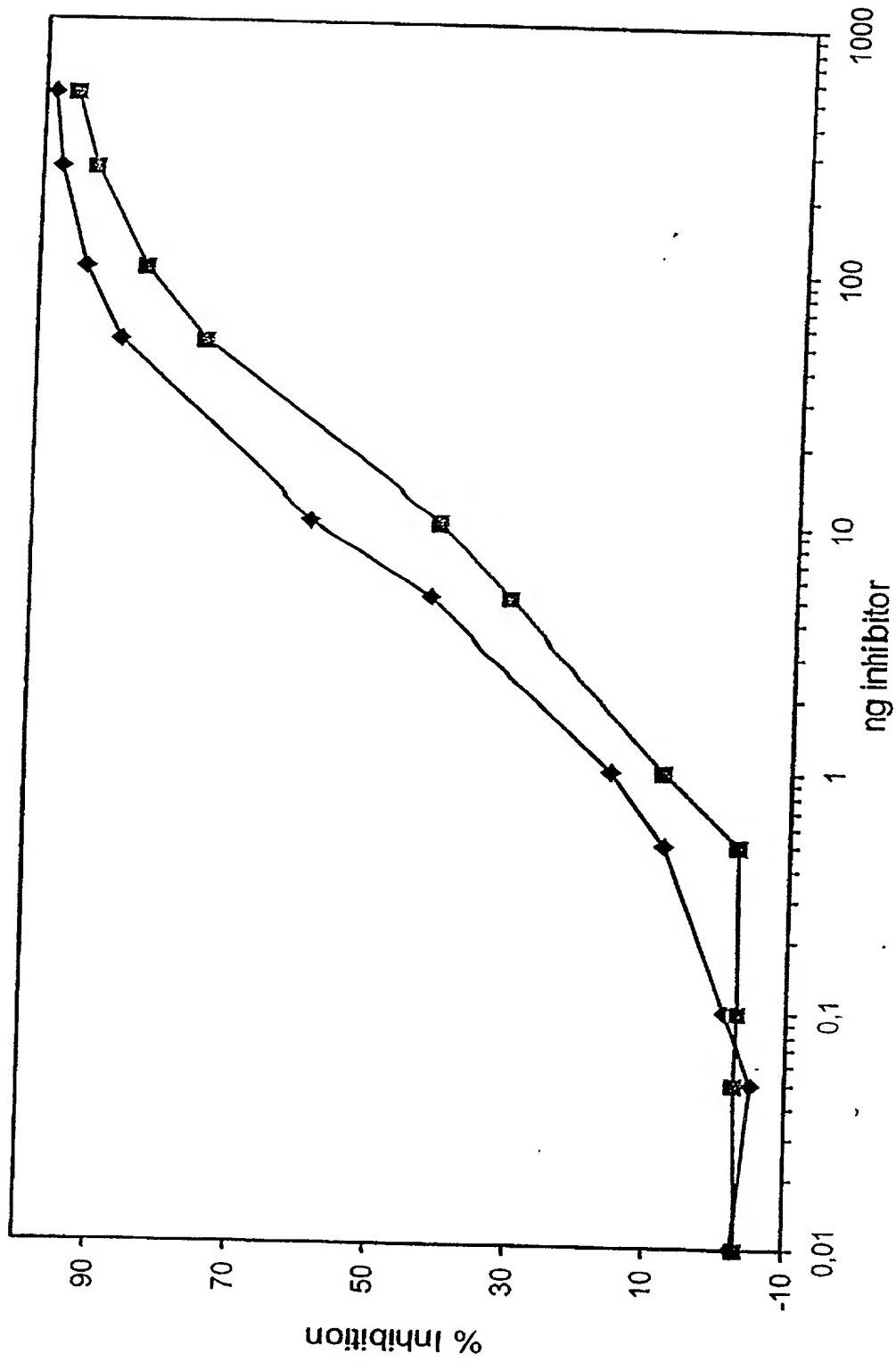


Fig. 6

—◆— Bet v 1 —■— Pro108Gly

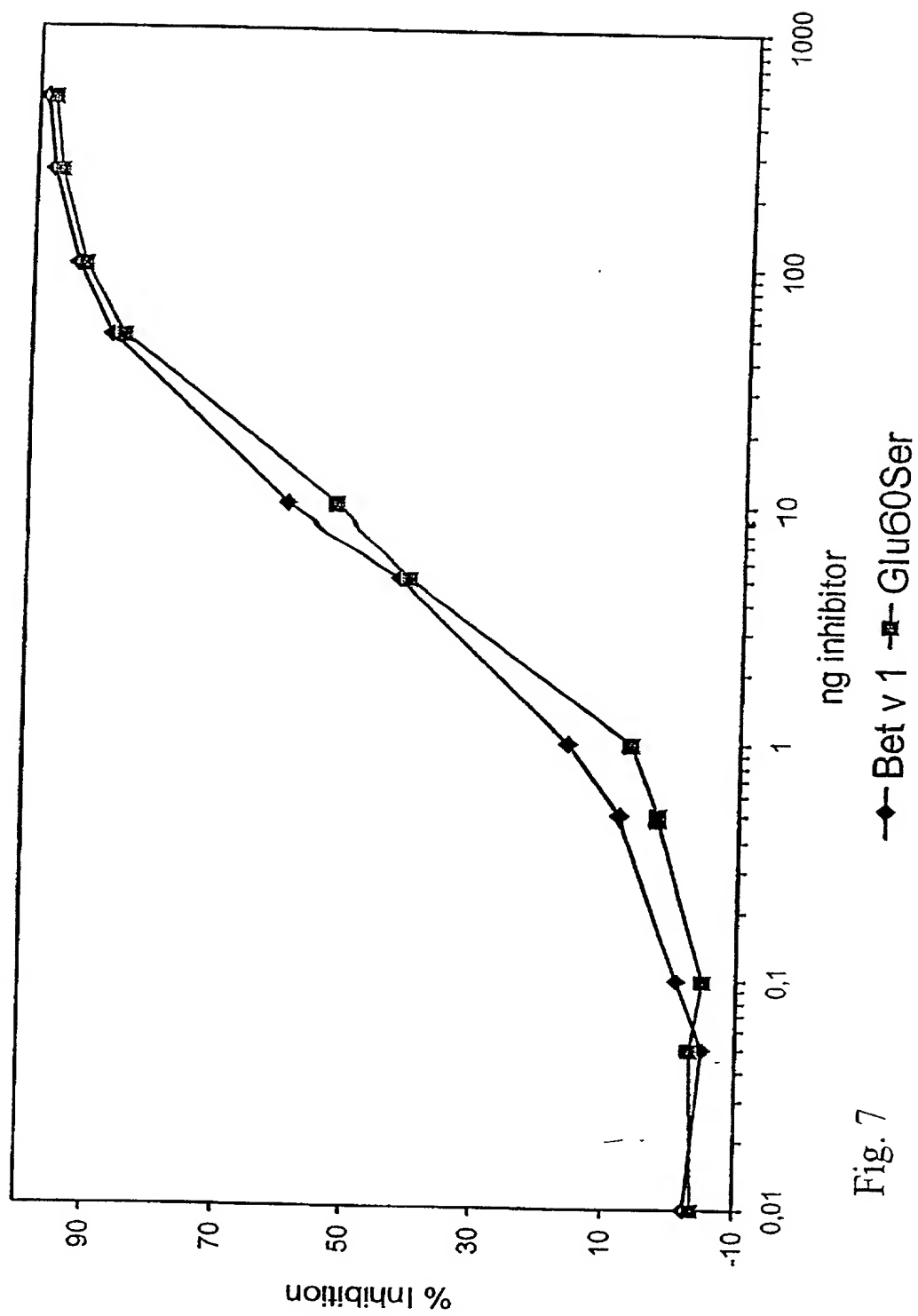


Fig. 7

10mM Na2HPO4/NaH2PO4 0, 02MNa3N3

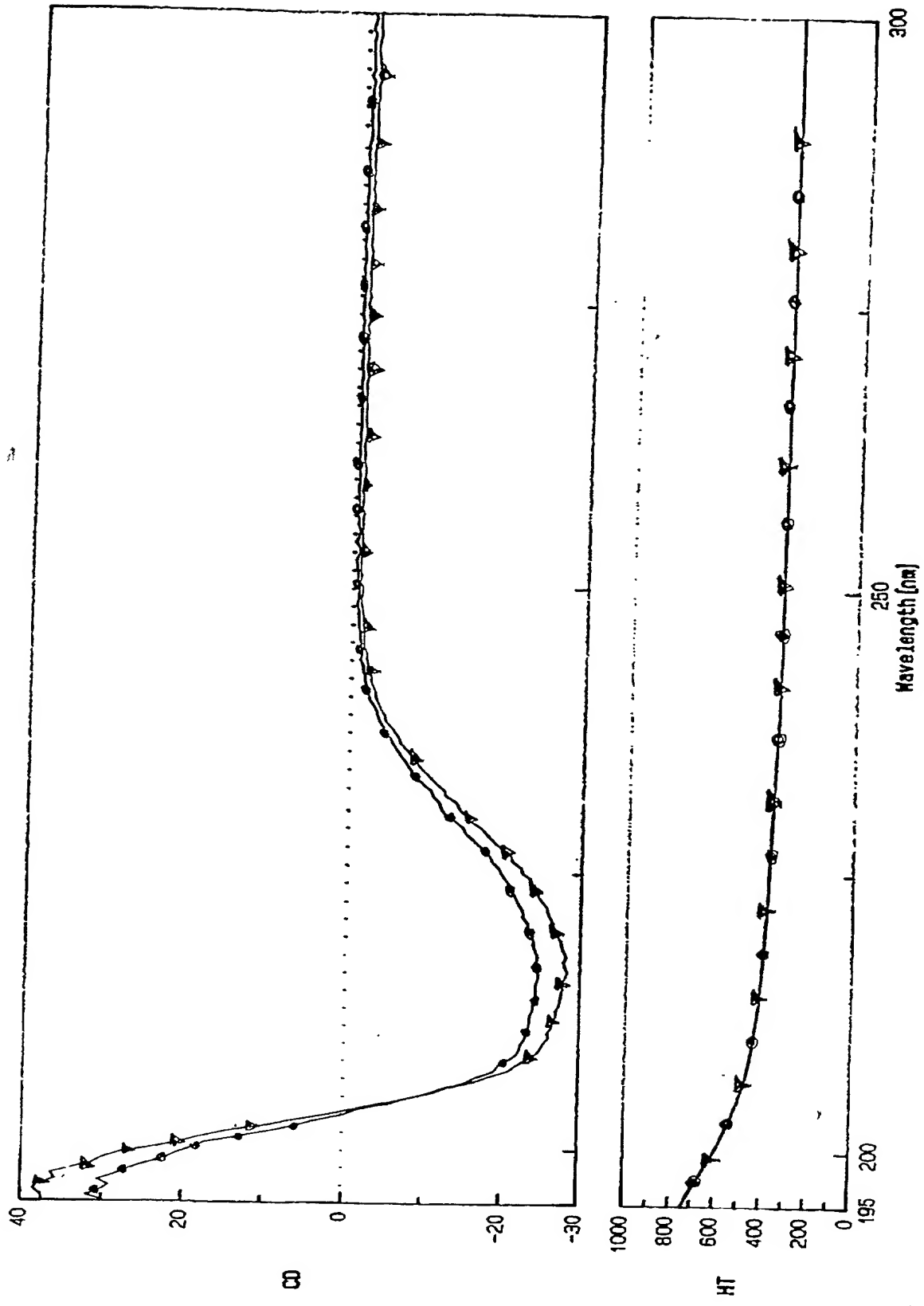
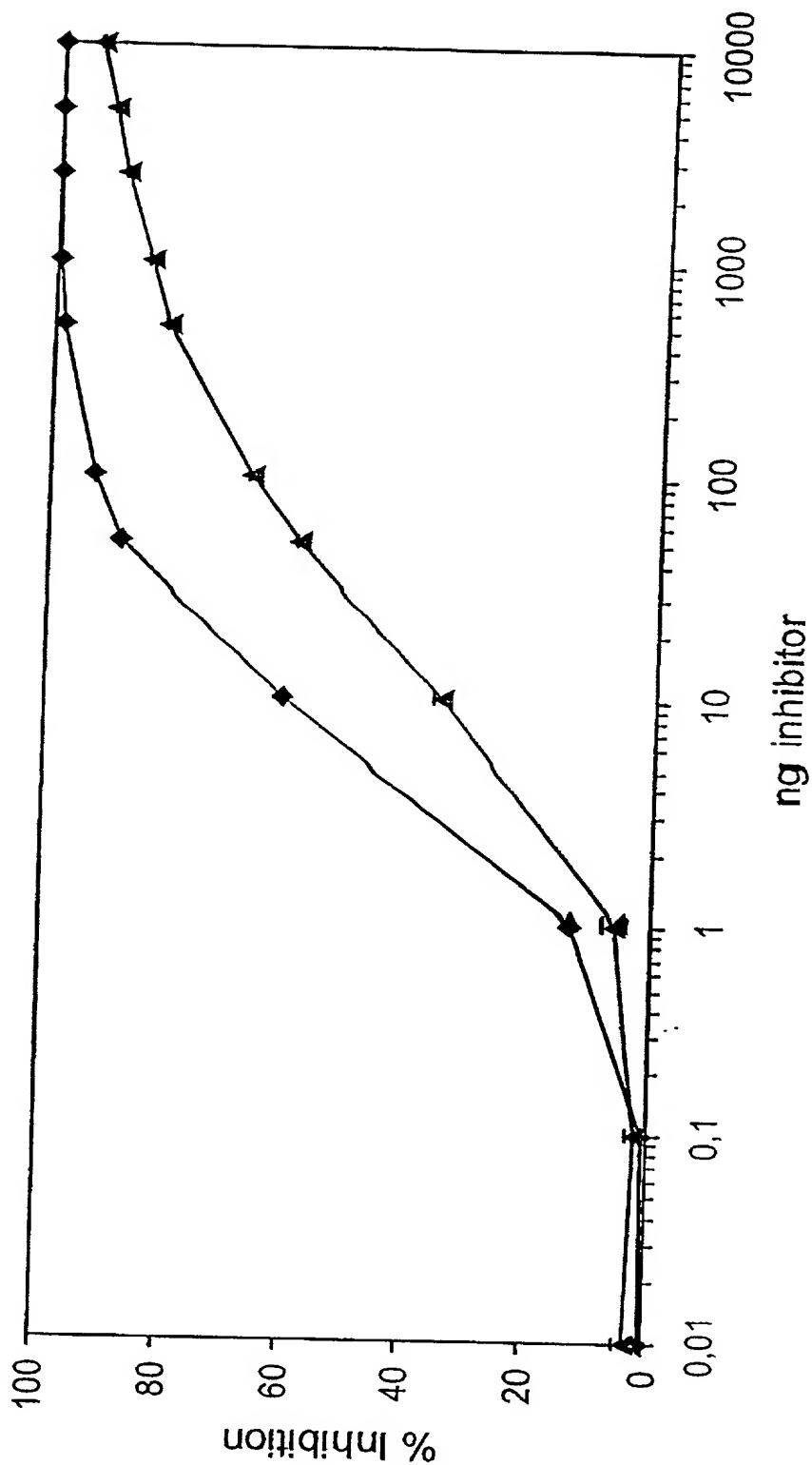


FIG. 8

— 5798a r Batv 2801  
 - - 5797a mutant 2595





◆ Bet v 1 ▲ Glu45Ser, Pro108Gly, Asn28Thr+Lys32Gln.

Fig. 9

Number of children	Percentage of families
0	10%
1	25%
2	35%
3	20%
4	10%
5	5%
6	2%

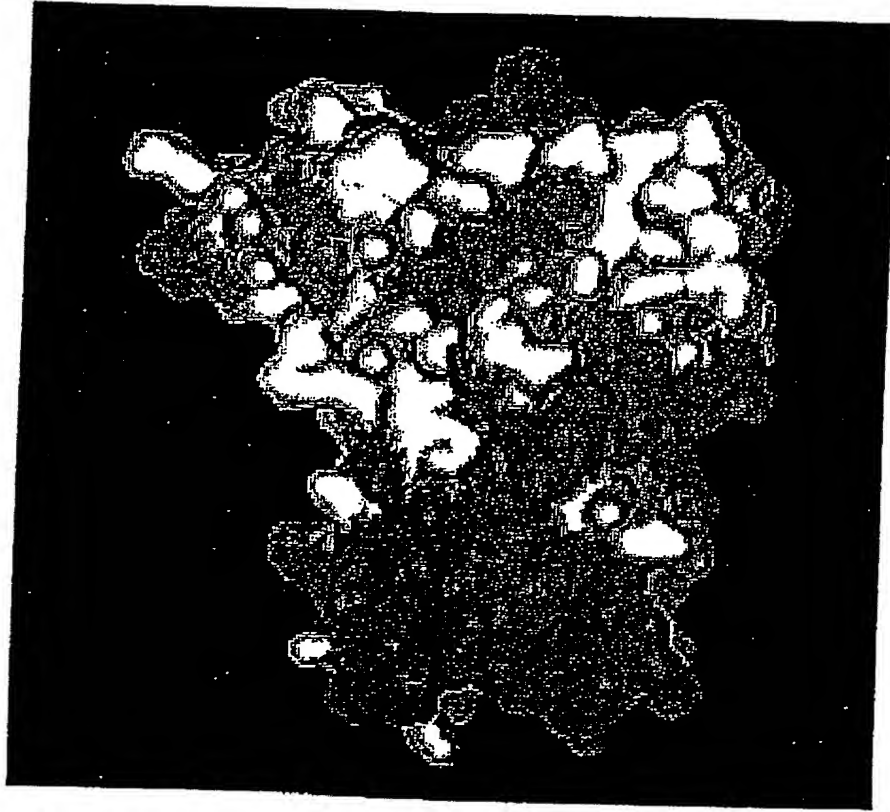
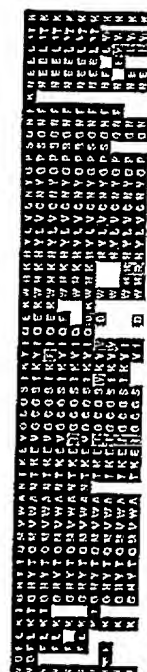
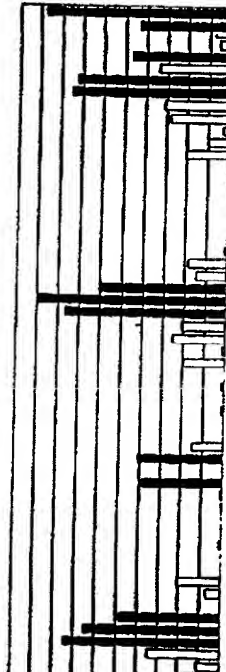
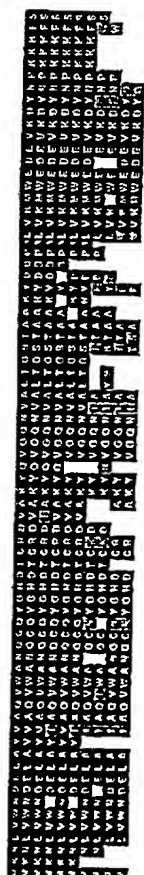
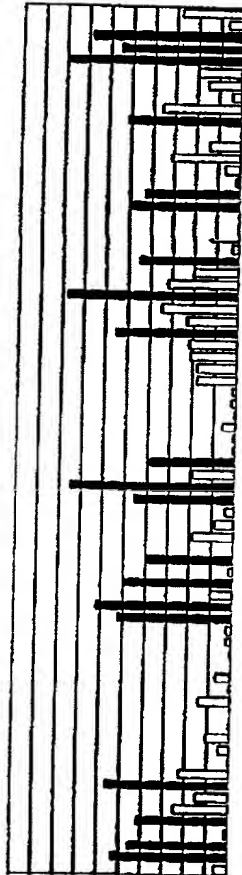
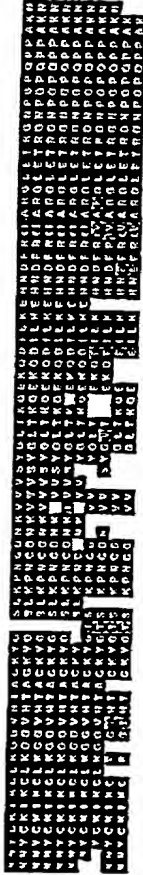


Figure 10.

Fig.11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.  
Mutated nucleotides underlined.

## Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5'-	ACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGA	-3'
Ves v 5 non-sense	3'-	TGGTGTTCGGAGGTCGCTTCTTATACTTTTAAACCATACCT	-5'
sense primer	5'-	CCAGCG <u>GCT</u> AATATGAAAAAT	-3'
non-sense primer	3'-	GTCGGAGGTCG <u>CGG</u> ATTATAC	-5'

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## Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5'-	GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG	-3'
Ves v 5 non-sense	3'-	CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCTAC	-5'
sense primer	5'-	TGTCAAG <u>CT</u> TGGTCACGATACT	-3'
non-sense primer	3'-	TTAGTTACAGTT <u>CG</u> ACCAGTG	-5'

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Fig. 12

12

Oligonucleotide primers for site directed mutagenesis of Ves v 5.

all sense 1: XhoI start, 38-mer:

EcoRI  
 5'-CCGCTCGAGAAAAGAAACAATTATTGTAAAATAAAATG  
     L E K R N N Y C K I K  
 Kex2 cleavage site amino terminus of Ves v 5

1	sense	1: K72As	21-mer	5'-CCAGCGGCTAATATGAAAAAT
1	non-sense	2: K72Aa	21-mer	5'-CATATTAGCCGCTGGAGGCTG
2	sense	3: Y96As	21-mer	5'-TGTCAAGCTGGTCACGATACT
2	non-sense	4: Y96Aa	21-mer	5'-GTGACCAGCTTGACATTGATT
all non-sense 7: CT-pPICZαA, 21-mer				5'-ATTCATCAGCTGCGAGATAGG

Fig. 13

13

## Overview of Ves v 5 mutations

1	AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA	60
1	N N Y C K I K C L K G G V H T A C K Y G	20
61	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG	120
21	S L K P N C G N K V V V S Y G L T K Q E	40
121	AAACAAGACATCTTAAAGGAGCACAAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG	180
41	K Q D I L K E H N D F R Q K I A R G L E	60
	1 [K72A] (AAG-GCT)	
181	ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAAATATGAAAAATTTGGTATGGAAC	240
61	T R G N P G P Q P P A K N M K N L V W N	80
	2 [Y96A] (TA-GC)	
241	GACGAGTTAGCTTATGTGCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT	300
81	D E L A Y V A Q V W A N Q C Q Y G H D T	100
301	TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT	360
101	C R D V A K Y Q V G Q N V A L T G S T A	120
361	GCTAAATACGATGATCCAGTTAAACTAGTTAAATGTGGGAAGATGAAGTGAAAGATTAT	420
121	A K Y D D P V K L V K M W E D E V K D Y	140
421	AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAAACGGGCCATTACACTCAAATG	480
141	N P K K K F S G N D F L K T G H Y T Q M	160
481	GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG	540
161	V W A N T K E V G C G S I K Y I Q E K W	180
541	CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT	600
181	H K H Y L V C N Y G P S G N F K N E E L	200
601	TATCAAACAAAGTAA	612
201	Y Q T K stop	204

100032454504

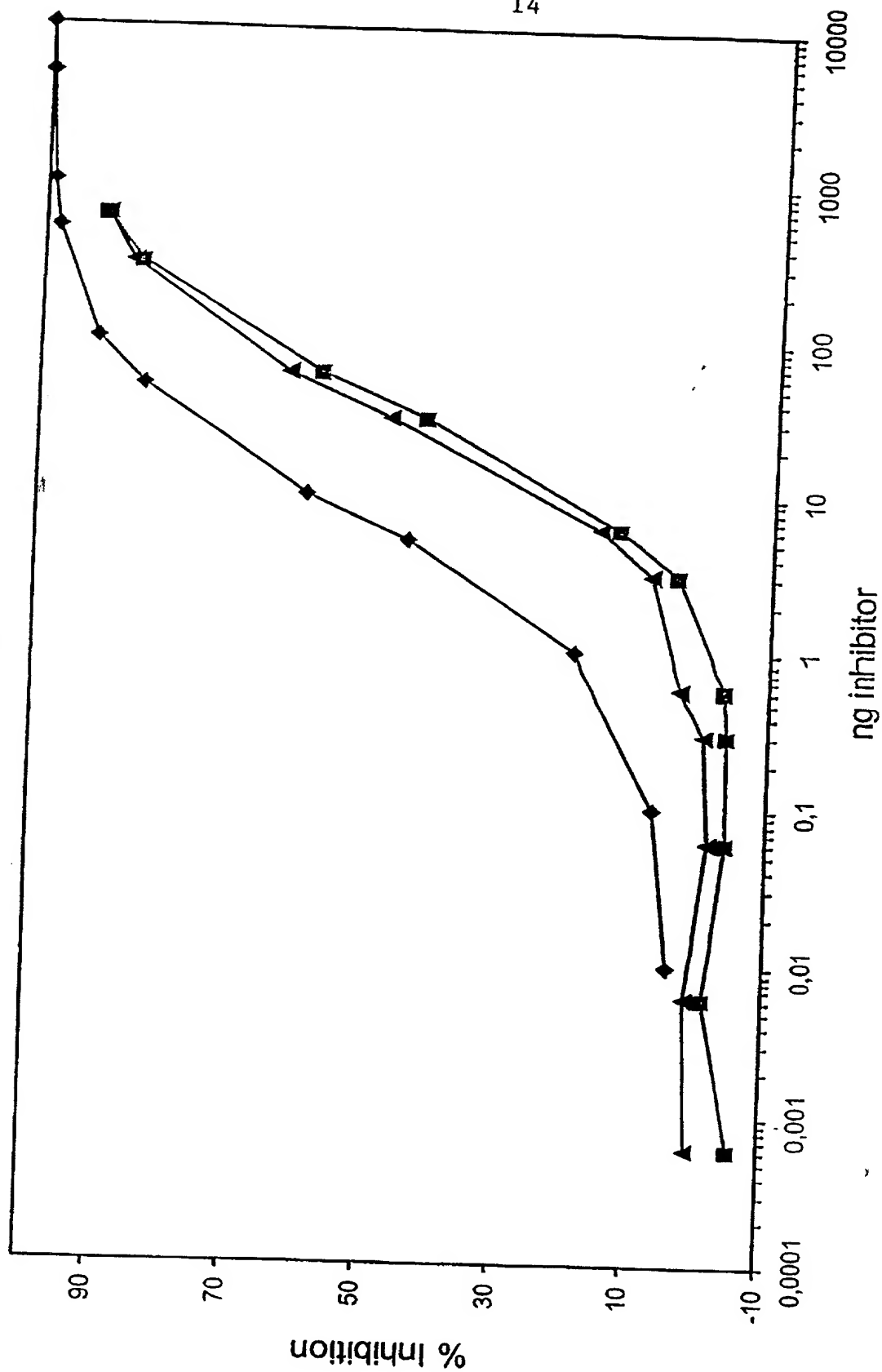
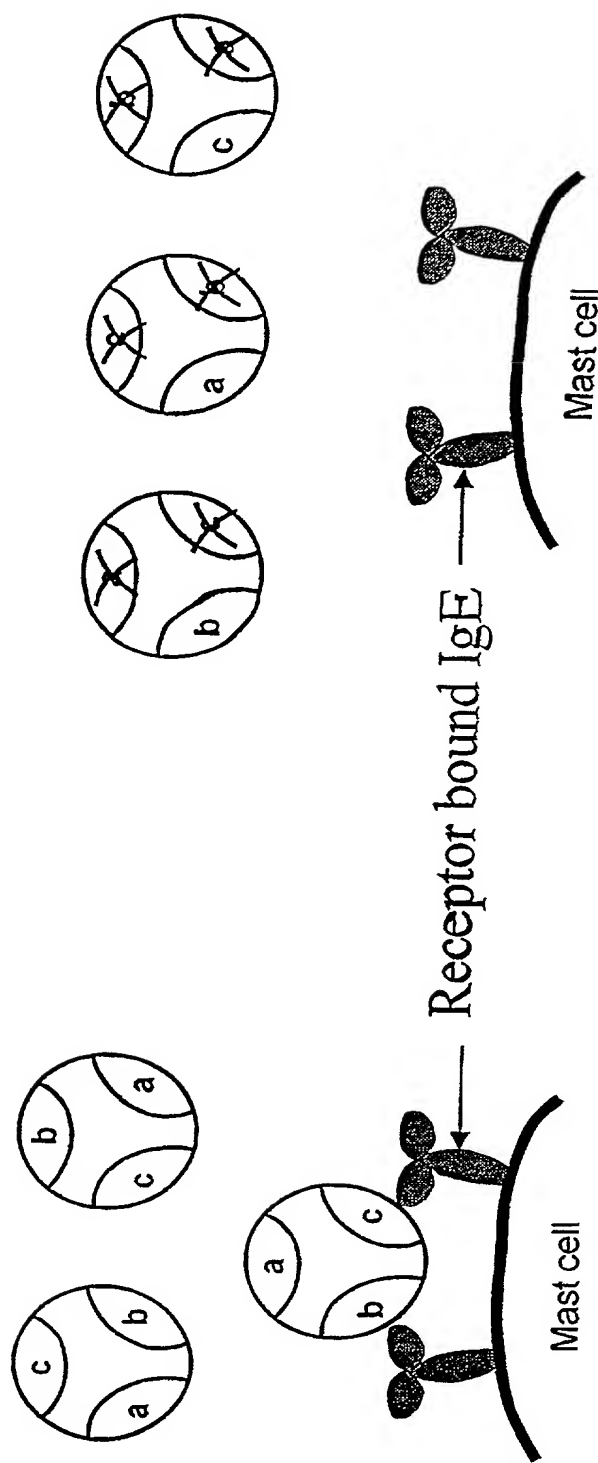


Fig. 14  
 —◆— r Ves v 5 —■— Lys72Ala —▲— Tyr96Ala

Fig. 15

Effect of point mutations in dominating IgE epitopes  
hypothetical model with 3 epitopes



Cross-linking

Fig. 15A

No cross-linking

Fig. 15B

Fig. 16

## DNA SEQUENCE

Der p 2 (DNA sequence referred to in notes in accession No. P49278 SWISSPROT)

## ORIGIN

```

1      cacaaattct ttttcttcc ttactactga tcattaatct gaaaacaaaa ccaaacaaac
61     cattcaaaat gatgtacaaa atttgtgtc ttcatgtt ggtcgcagcc gttgctcgtg
121    atcaagtoga tgtcaaagat tgtgccaatc atgaaatcaa aaaagtttg gtaccaggat
181    gccatgggtc agaaccatgt atcattcatc gtggtaaacc attccaattg gaagccgttt
241    tcgaagocaa ccaaaacaca aaaacggcta aaattgaaat caaagcctca atcgatggtt
301    tagaagttga tgttccoggt atcgatccaa atgcatgcca ttacatgaaa tgcccattgg
361    ttaaaggaca acaatatgat attaaatata catggaatgt tccgaaaatt gcaccaaaat
421    ctgaaaatgt tgtcgtcact gttaaagtta tgggtgatga tgggtgtttg gcctgtgcta
481    ttgtactca tgctaaaatc cgcgattaaa tcaaacaaaa ttattgatt ttgtaatcac
541    aaatgattga ttttcttcc aaaaaaaaaa taaataaaat ttgggaatt c

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## AMINO ACID SEQUENCE

Der p 2 (Accession No. P49278 SWISSPROT; includes signal peptide 1-17)

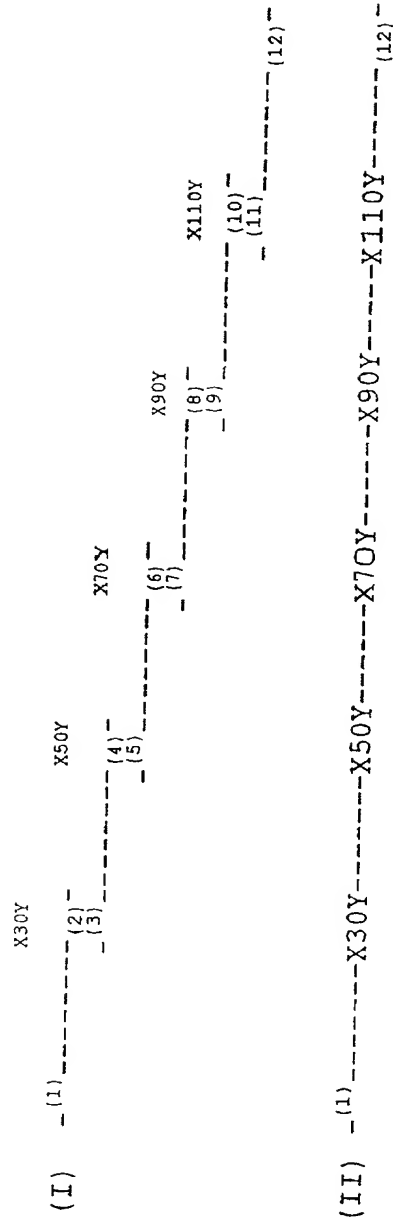
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1      mmykilclsl lvaavardqv dvkdcanhei kkvlvpchg sepciihrk pfqleavfea
61     nqntktakie ikasidglev dvpgidpnac hymkcplvkg qqydikytwn vpkiapksen
121    vvvtkvmgd dgvlacaiat hakird

```



Figure 17



Lines represents DNA sequences.

Numbers in parentheses above lines represents sense oligonucleotide primers: (1), (3), (5), (7), (9), (11).  
 Numbers in parentheses below lines represents anti-sense oligonucleotide primers: (2), (4), (6), (8), (10), (12).  
 Notation X (position) Y represents mutations.

(1) Represents the sense oligonucleotide primer accommodating the protein N-terminus.

(12) Represents the anti-sense oligonucleotide primer accommodating the protein C-terminus.

Figure 18

Bet v 1 (2628) (Y5V, E45S, K65N, K97S, K134E)

DNA template: Bet v 1 (2589) carrying the Y5V mutation.

331pMalc (s)  
189BV (a)  
188BV (s)  
362BV (a)  
361BV (s)  
364BV (a)  
363BV (s)  
366BV (a)  
365BV (s)  
332pMalc (a)

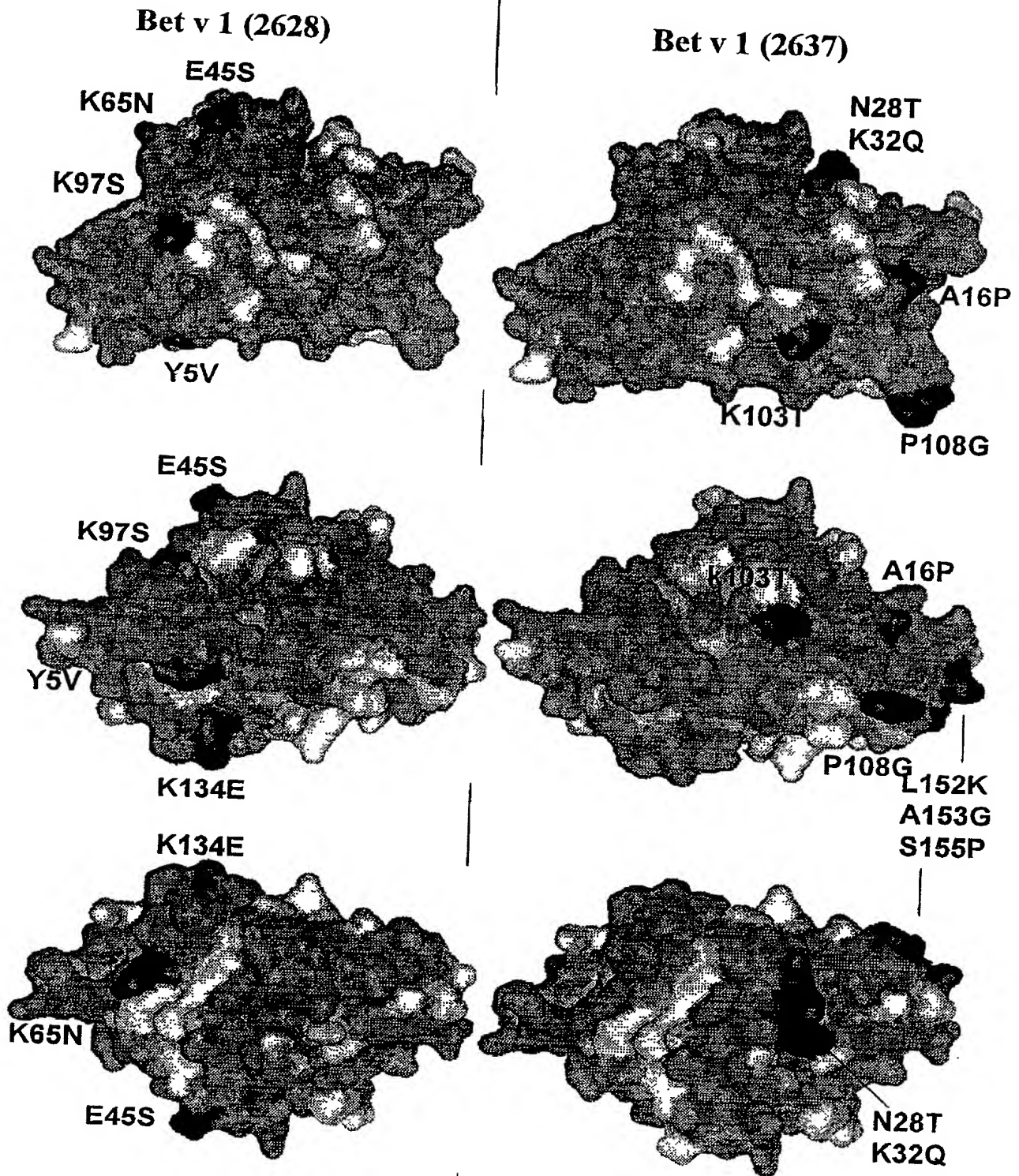
331pMal c : CAGACTAATTTCGAGCTCGGTACCC  
189BV : TTTCCTGAAATGTTTCAACACT  
188BV : AACATTCAGGAAATGGAGGGCC  
362Bva : CACGTAGTTGAAAGGGAGGCCTTC  
361BV s : TTCAACTACGTGAAGGACAGAGT  
364Bva : GGAGATGCTCTCCAATGTGTCGCC  
363BV s : GGAGAGCATCTCCAACGAGATAAA  
366Bva : ACTTGCTTCAACCTGCTCTGCCTT  
365BV s : CAGGTTGAAGCAAGTAAAGAAATG  
332pMal c : GCAGGTCGACTCTAGAGGATCCAT

Bet v 1 (2637)

(A16P, N28T, K32Q, K103T, P108G, L152K, A153G, S155P)

DNA template: Bet v 1 (2571) carrying N28T, K32Q, P108G mutations.

331pMalc  
368Bva  
367BV s  
370Bva  
369BV s  
372Bva  
331pMalc : CAGACTAATTTCGAGCTCGGTACCC  
368Bva : CAGTCGcggtGCTGGGATAACAGA  
367BV s : CCAGCACcgCGACTGTTCAAGGCC  
370Bva : CACTATggtTATCTCGTTGGAGAT  
369BV s : GAGATAaccATAGTGGCAACtggT  
372Bva: TTACTGAATTCATTAGTTGTAGGCATCcggtGgcctttGAGGTA



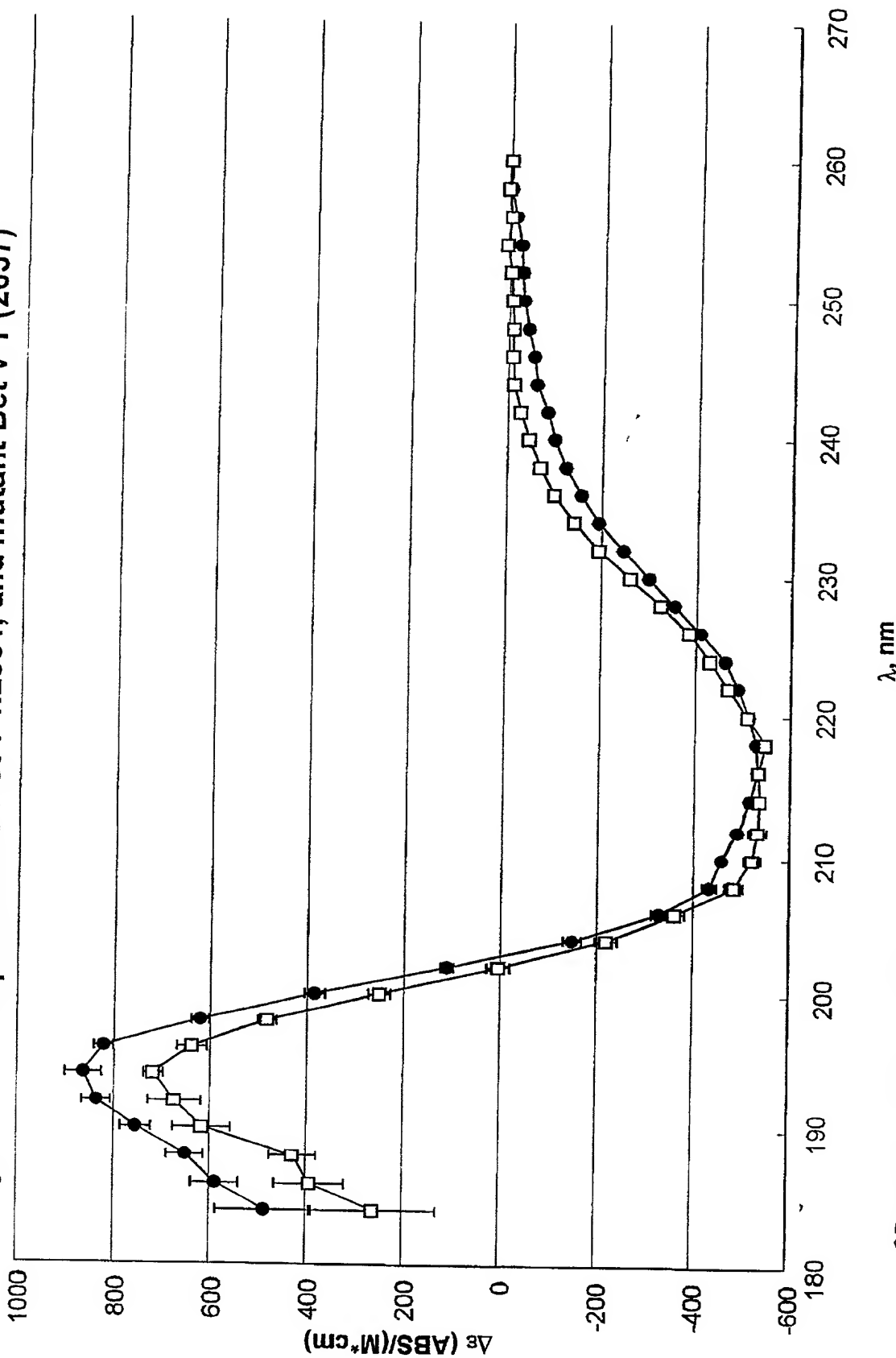
Molecular surface of Bet v 1.

Left side: Bet v 1 (2628), Right side: Bet v 1 (2637)

Grey: Backbone + amino acids 95-100% conserved among *Fagales*  
 Black: Introduced point mutations.

Figure 19

Figure 20 CD-spectrum of Bet v 1.2801, and mutant Bet v 1 (2637)



CD-spectrum of Bet v 1 (2637), open squares, and the CD-spectrum of native folded Bet v 1.2801, closed circles, both obtained at 20 °C

**Inhibition of human serum IgE-binding to Bet v 1.2801  
with Bet v 1.2801 and mutated Bet v 1 allergens**

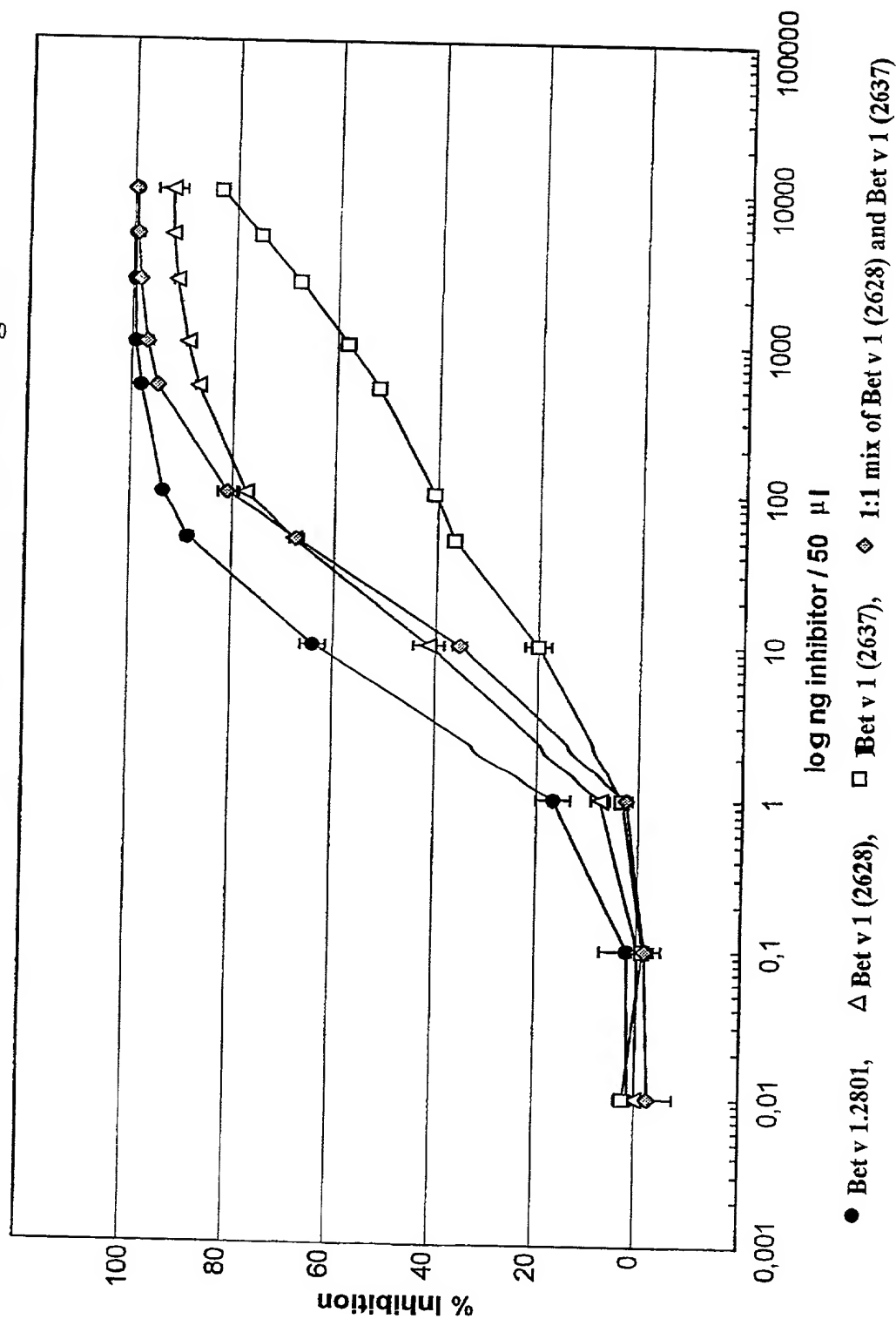


Figure 22 Histamine release, donor MCDS, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)

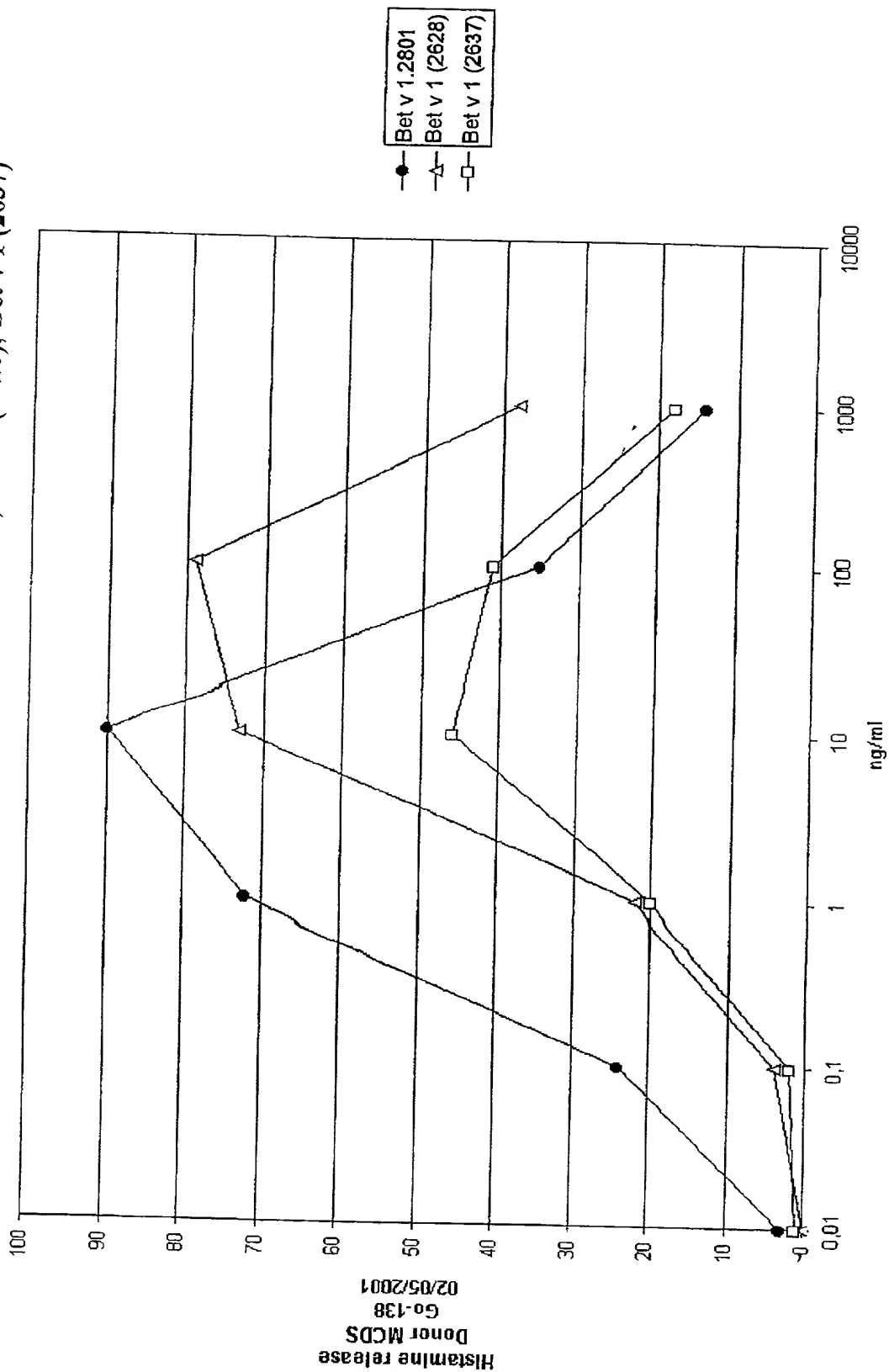


Figure 23 Histamine release, donor MDH, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)

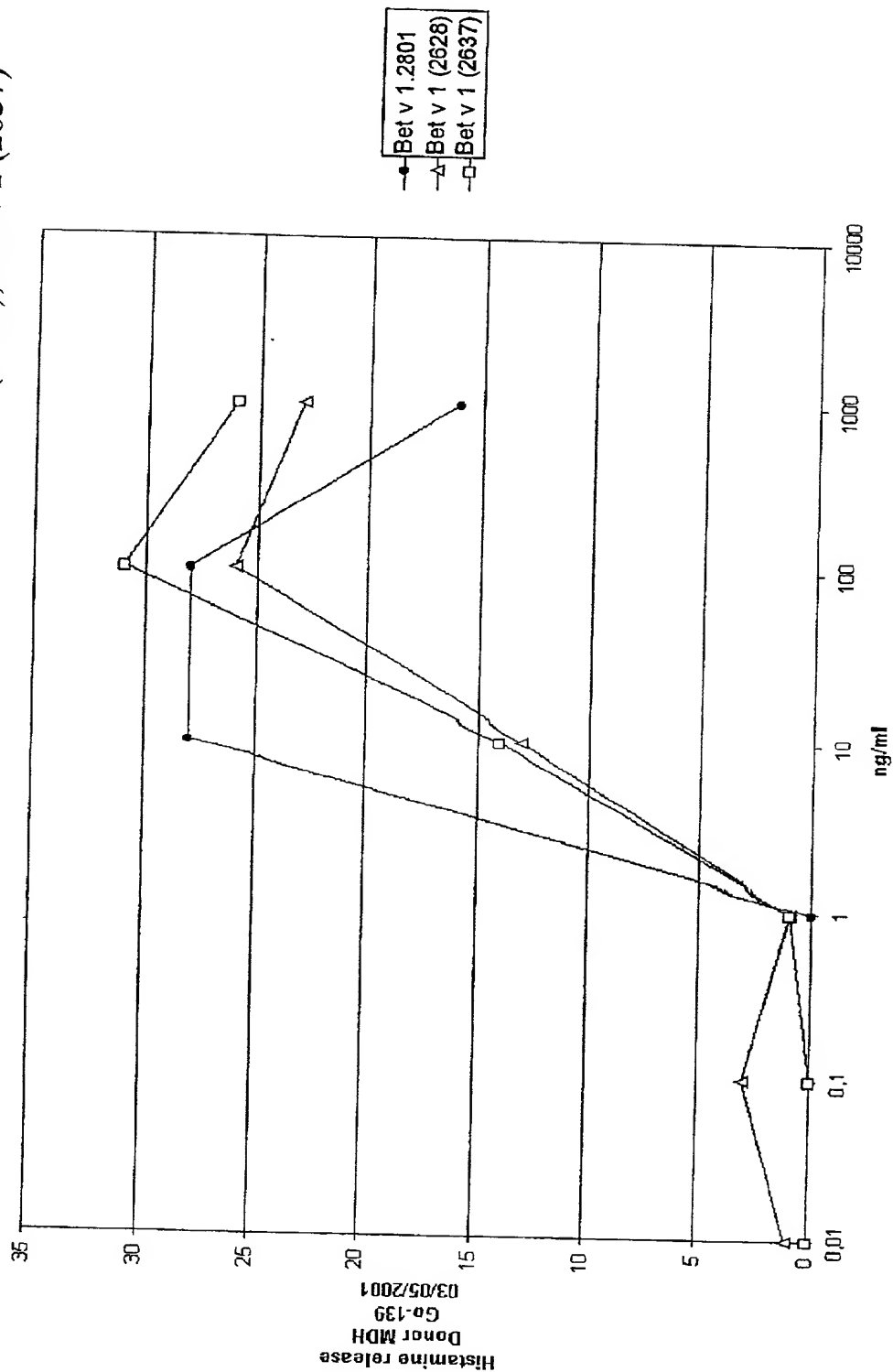
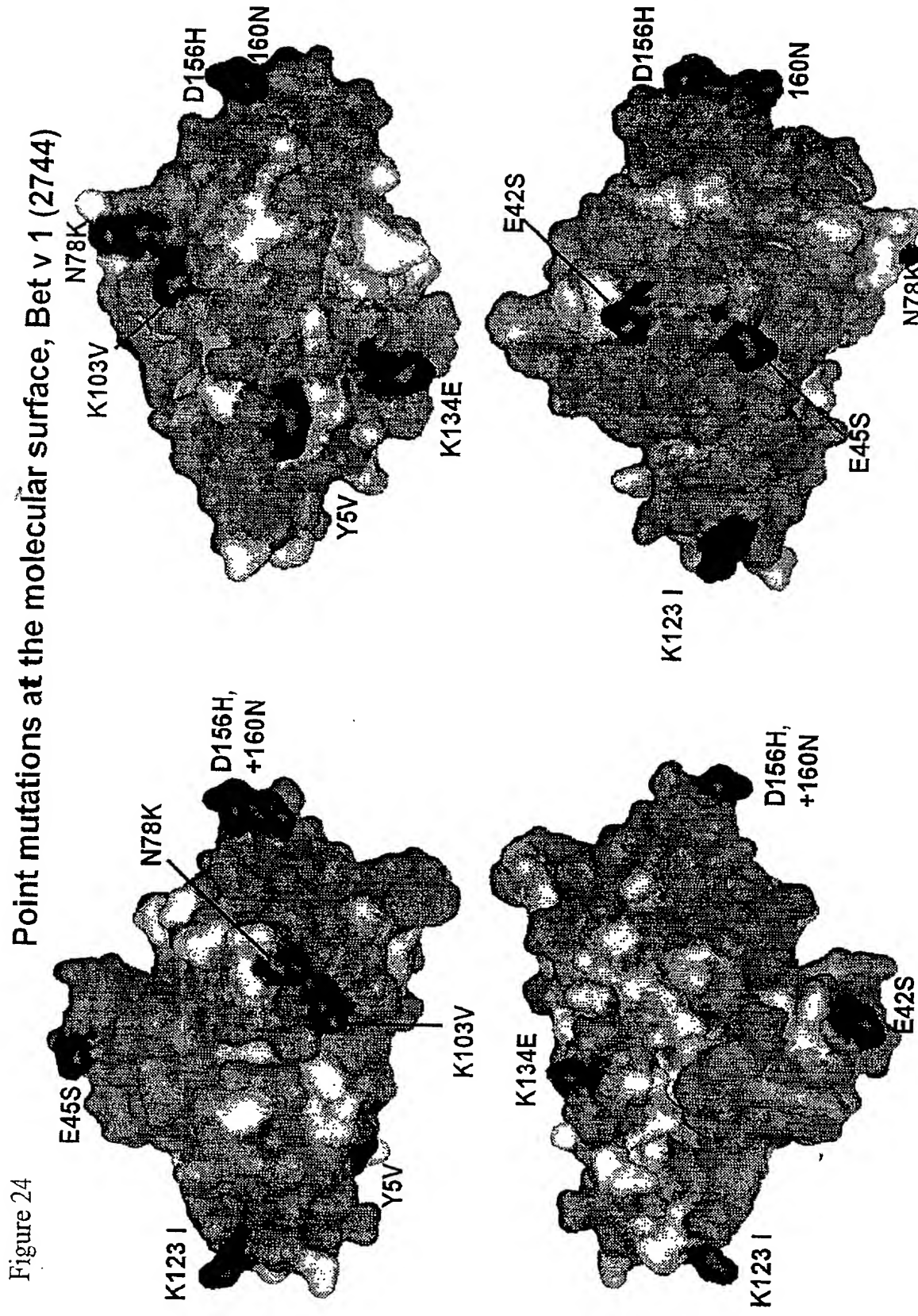


Figure 24

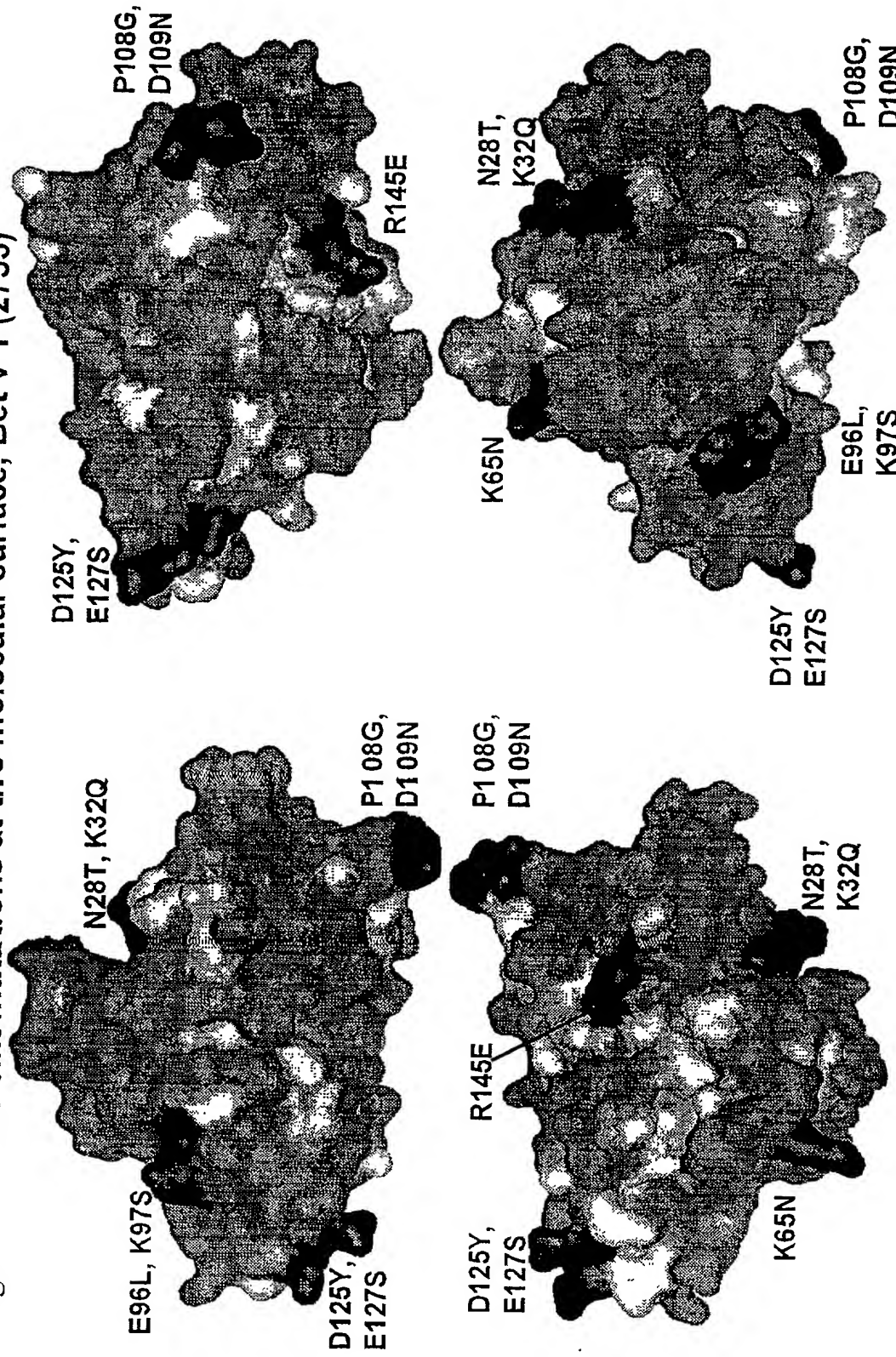


Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*, Black: Point mutations



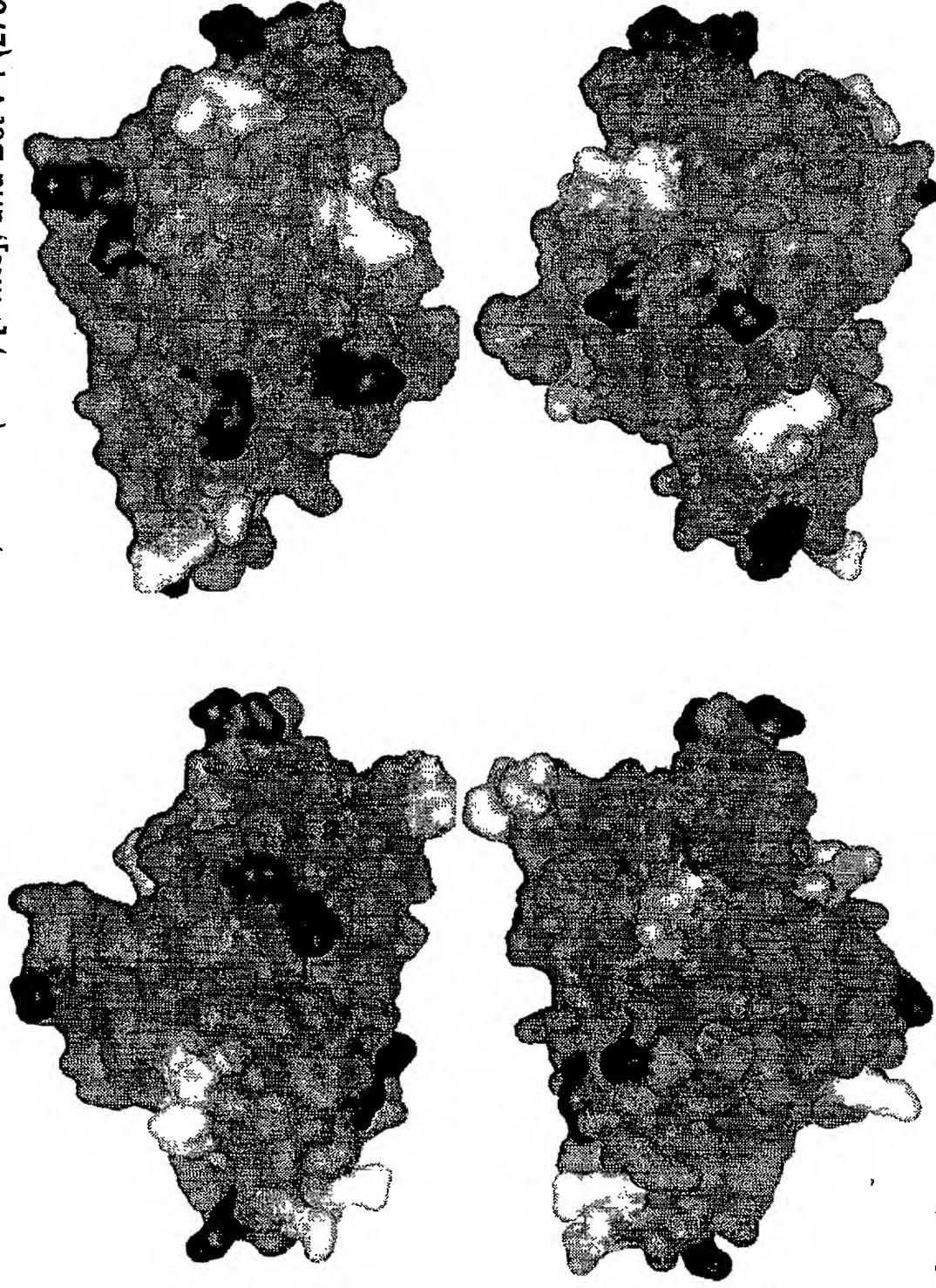
Figure 25

Point mutations at the molecular surface, Bet v 1 (2753)



Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*, Black: Point mutations

Distribution of point mutations at the molecular surface of, Bet v 1 (2744) [white], and Bet v 1 (2753) [Black]



Grey: Molecular surface; amino acid residues 95-100% conserved among *Fagales*  
 Black: Mutations (Y5V, K134E), (E42S, E45S), (N78K, K103V), K123 I, (D156H, +160N)  
 White: Mutations (N28T, K32Q), K65N, (E96L, K97S), (P108G, D109N), (D125Y, E127S), R145E

Figure 26

Figure 27 Circular dichroism spectra of Bet v 1.2801 and mutant Bet v 1(2744), pH 7.13, T 20C.

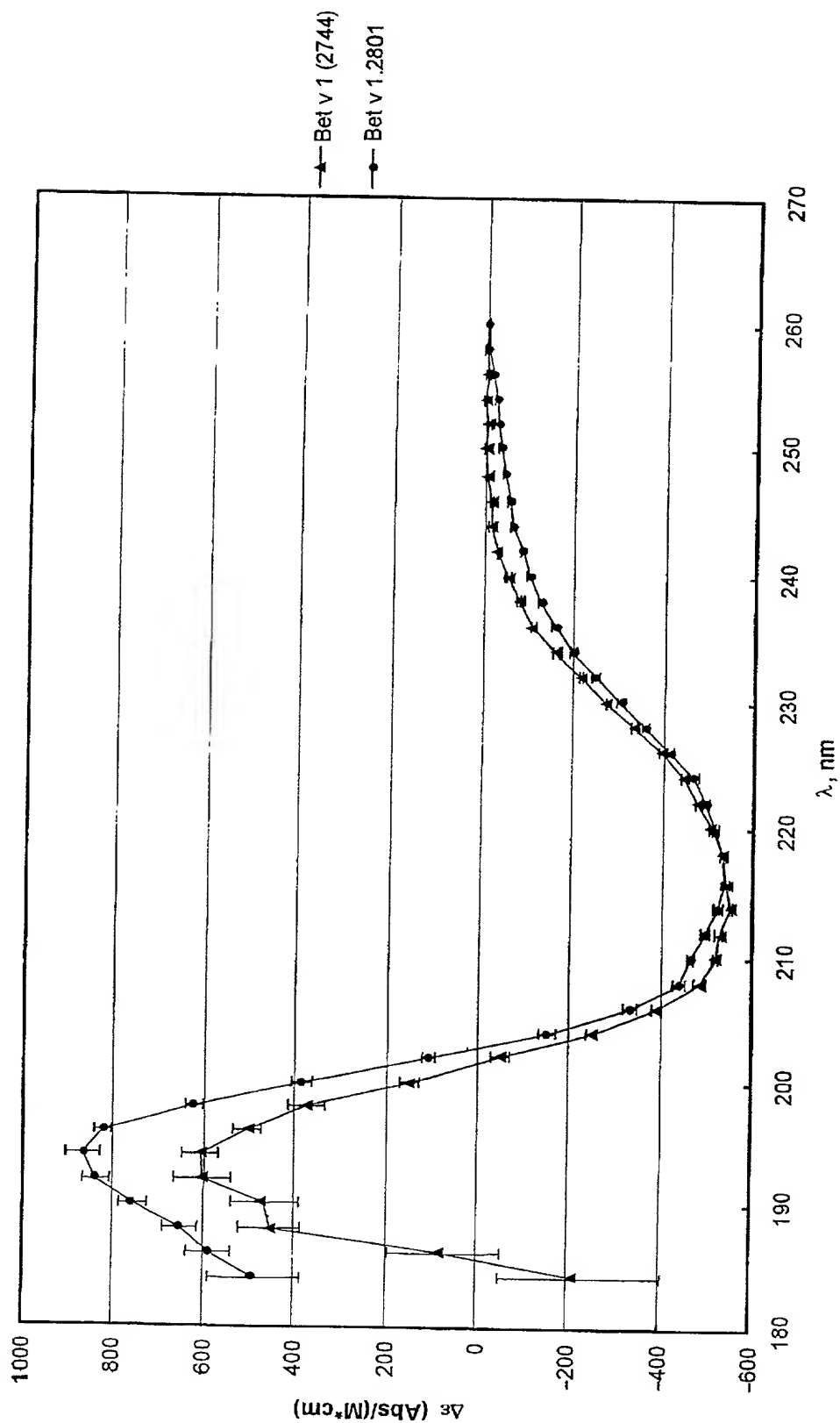
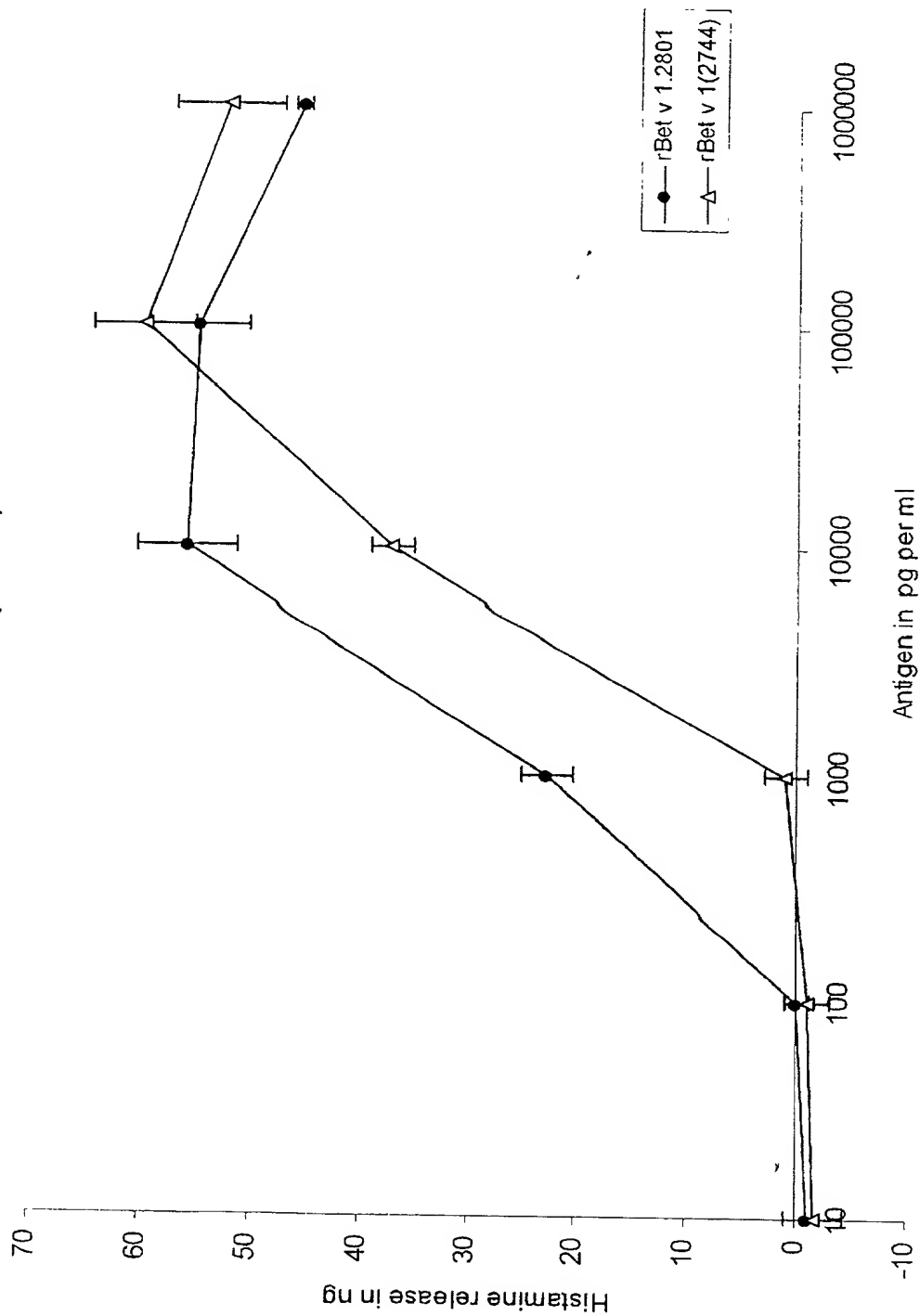


Fig. 28: Histamine release, donor MK, Bet v 1.2801 and Bet v 1(2744)



**Fig. 29A: Histamine release, donor MJ, Bet v 1.2801 and Bet v 1 (2744)**

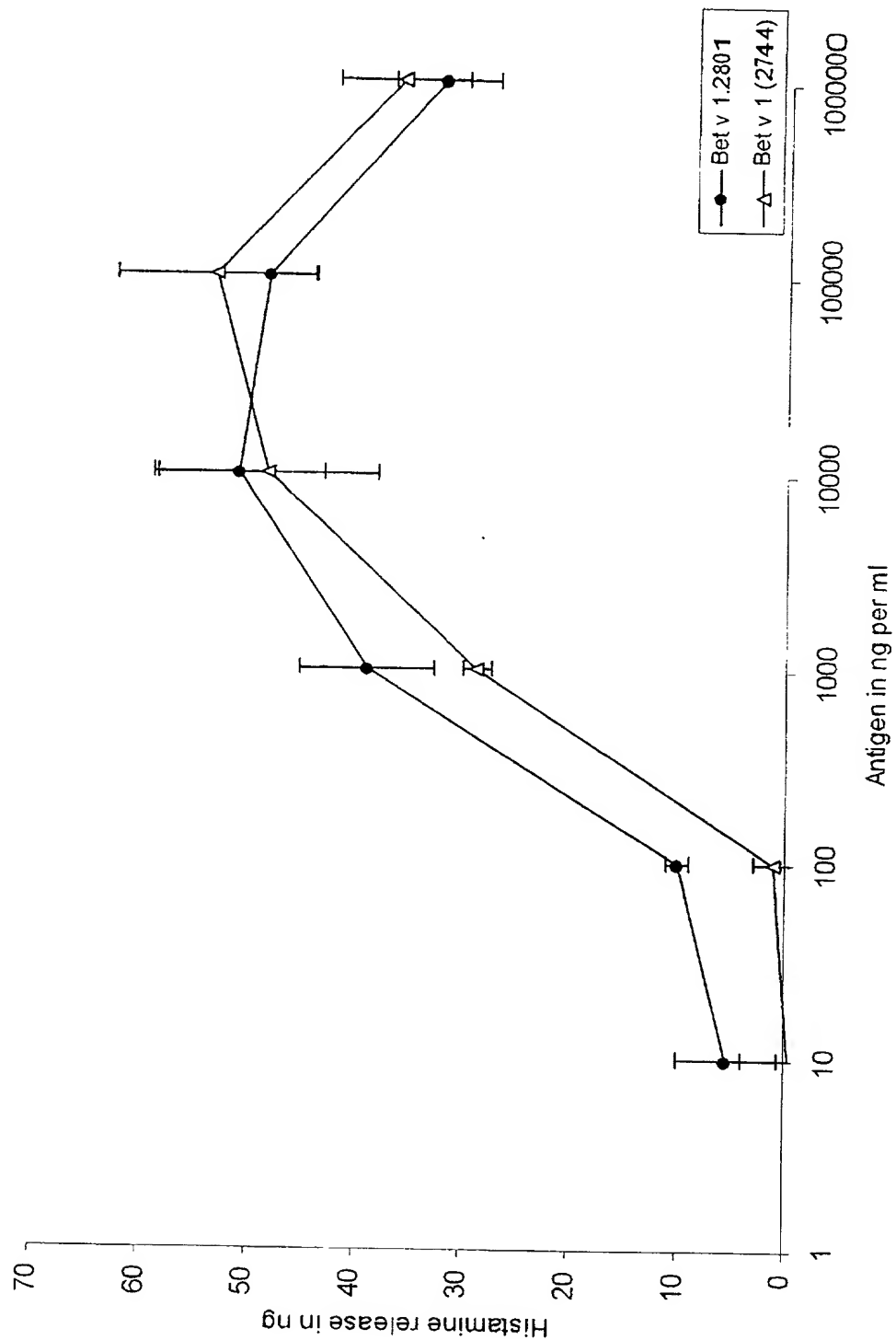


Fig.29B: Histamine release, donor MH, Bet v 1.2801 and Bet v 1 (2744)

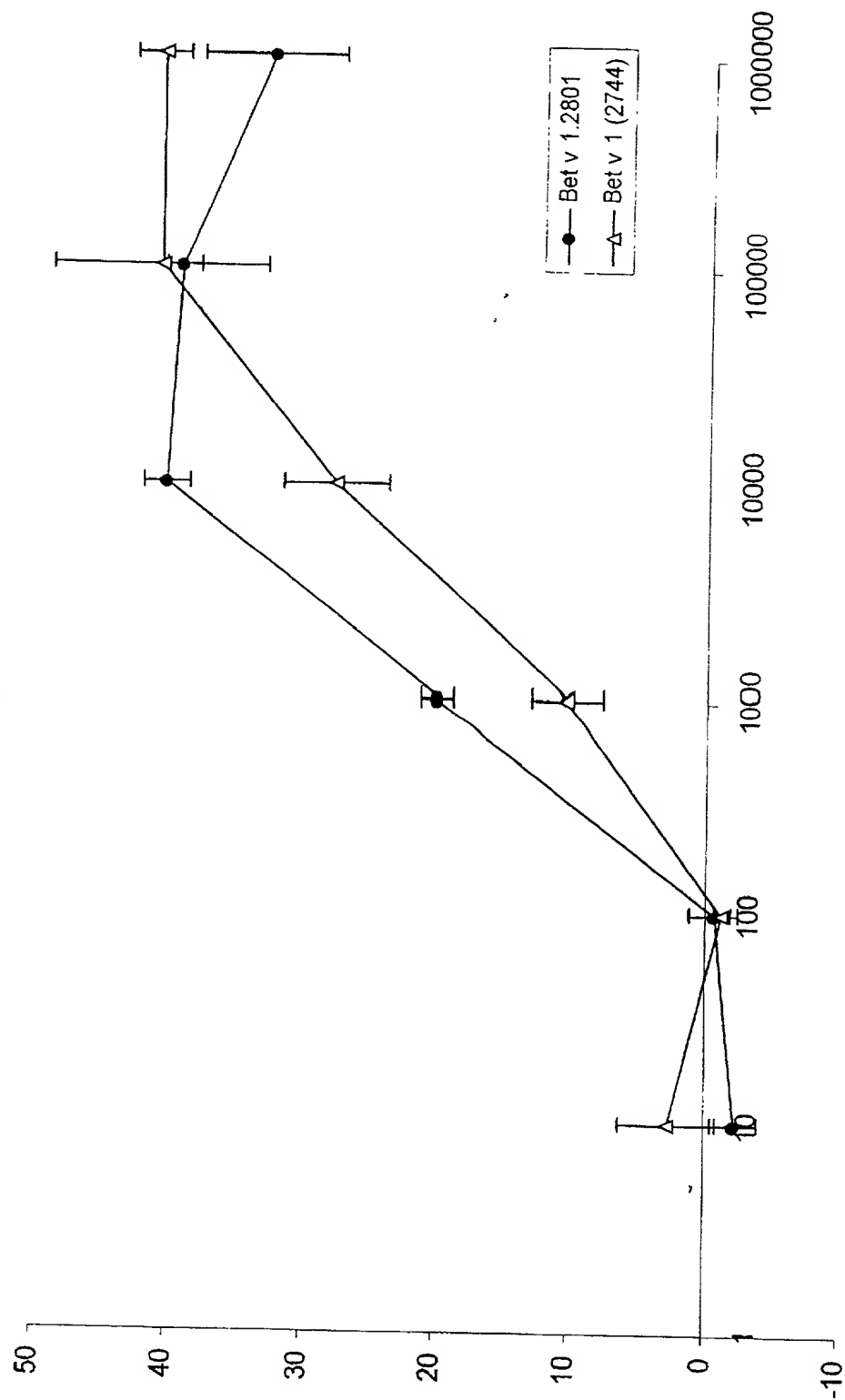


Fig. 29C: Histamine release, donor CJB, Bet v 1.2801 and Bet v 1 (2744)

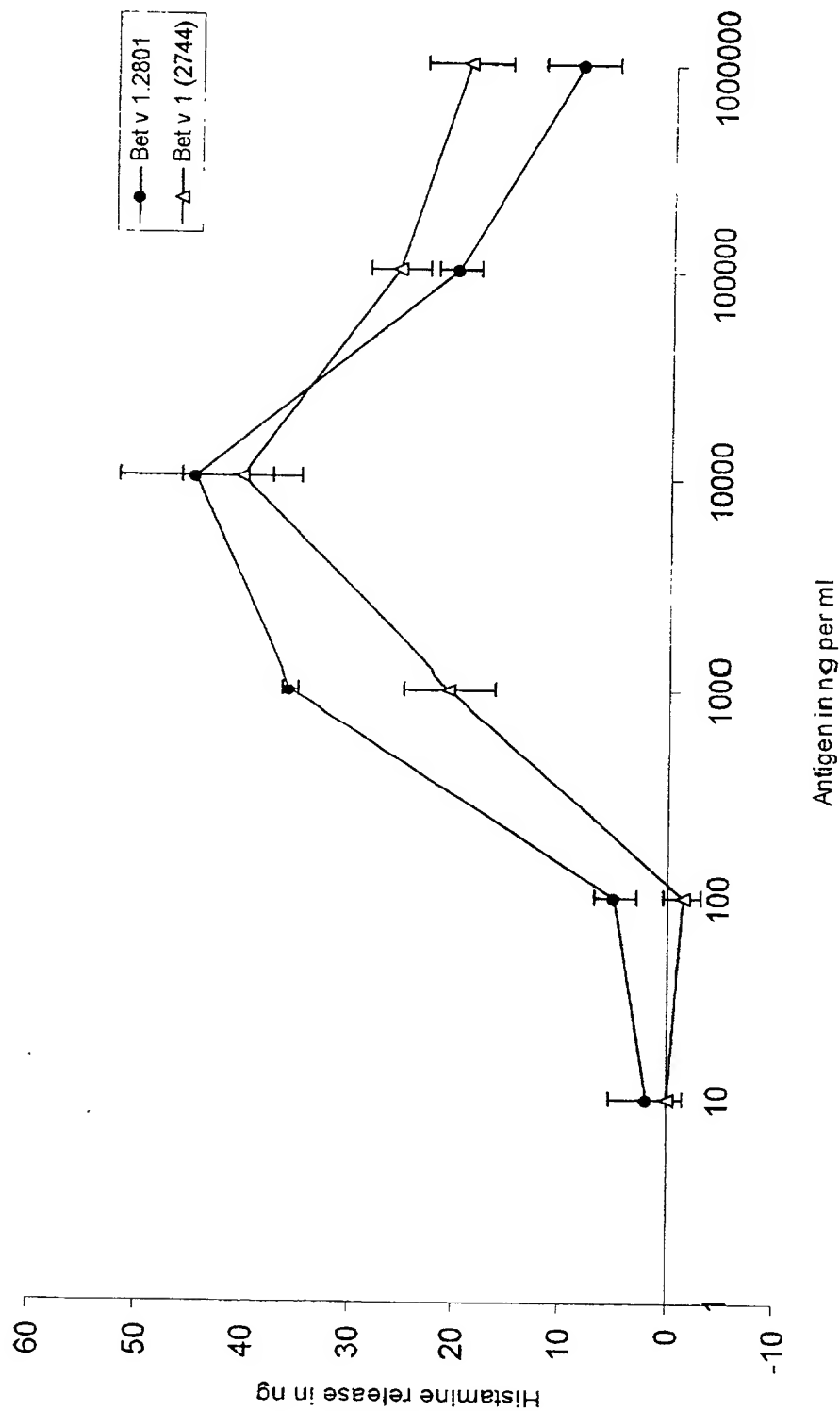


Fig. 29D: Histamine release, donor MCDS, Bet v 1.2801 and Bet v 1 (2744)

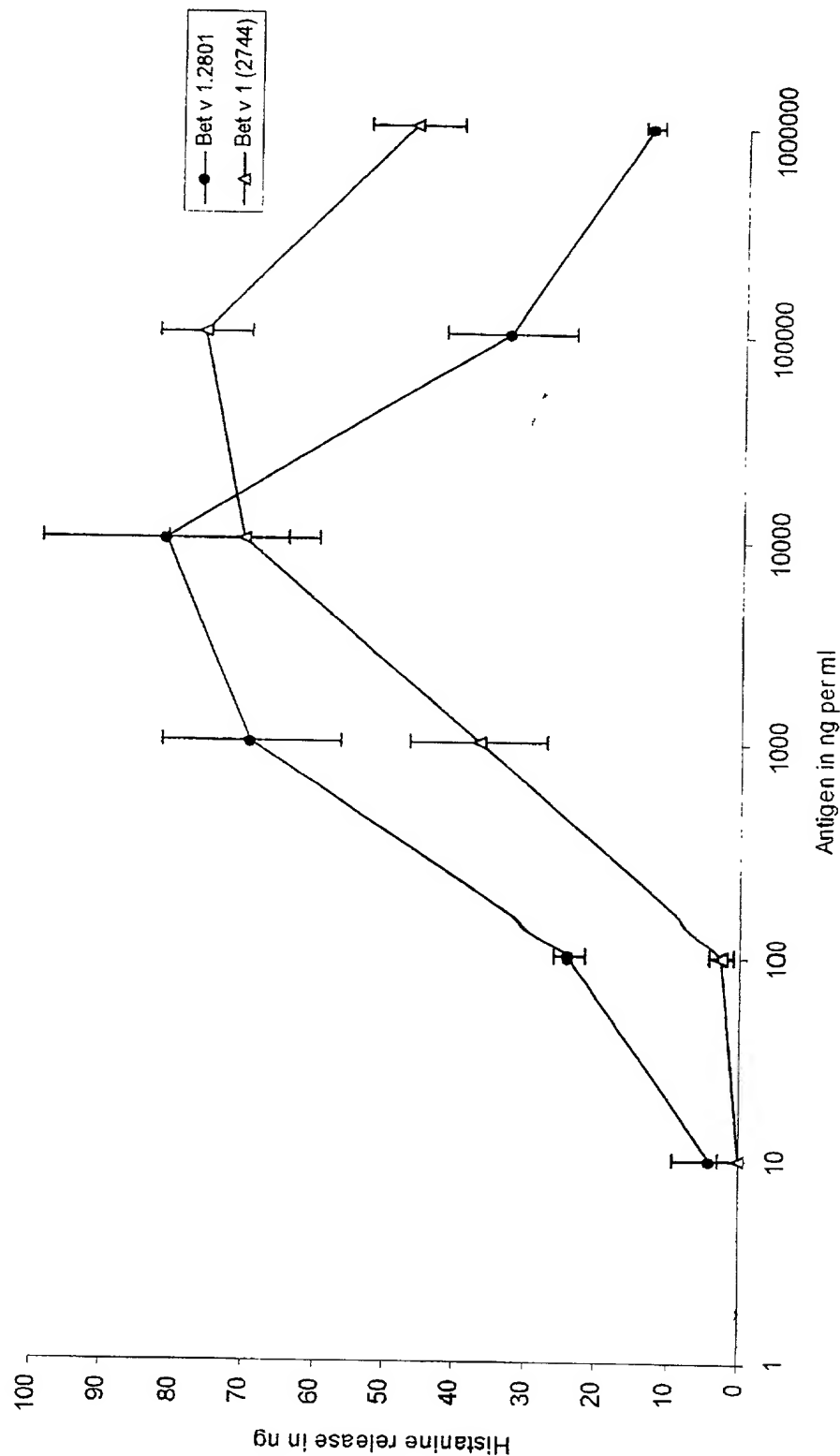
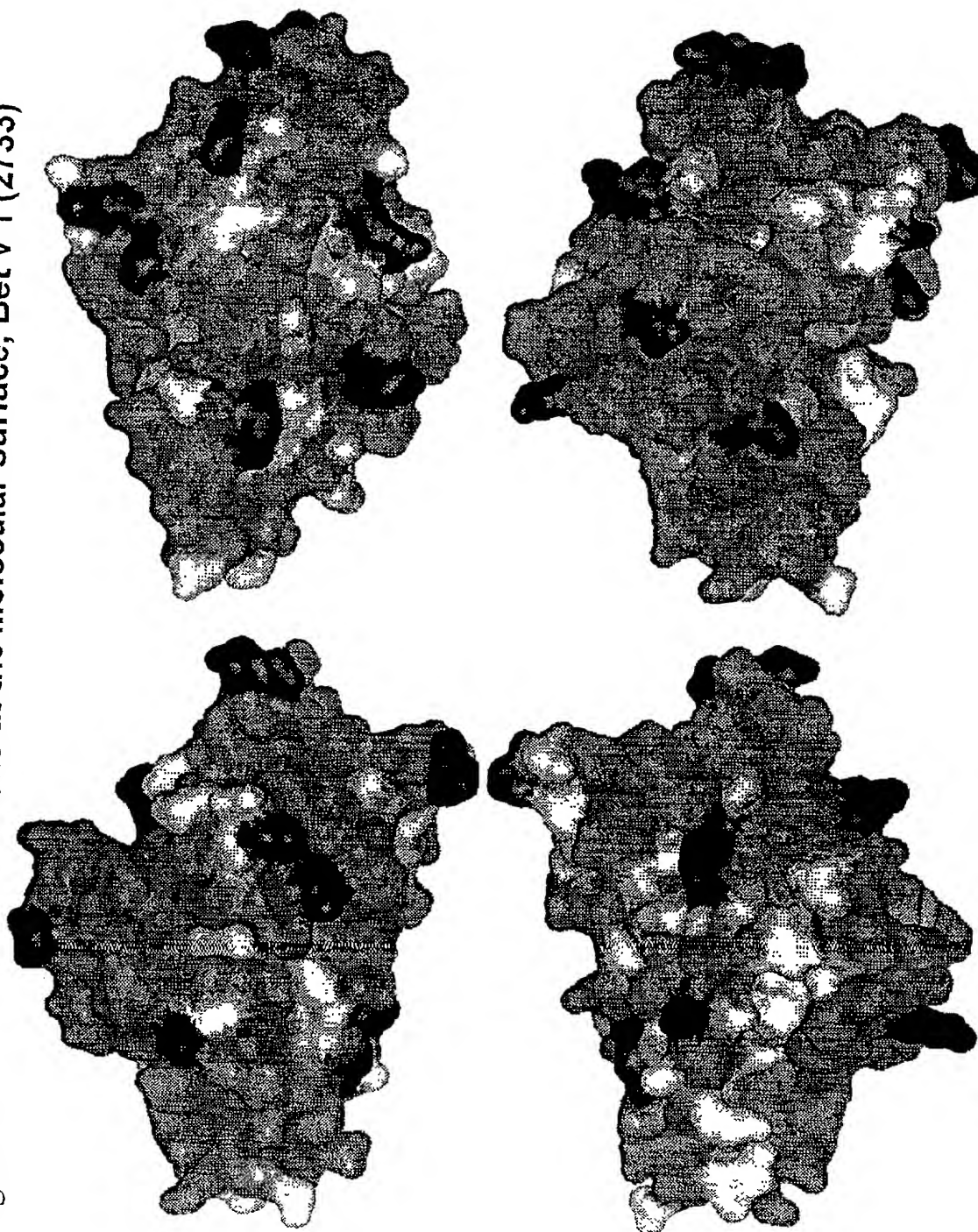




Figure 30 Point mutations at the Molecular surface, Bet v 1 (2733)



Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*,  
Black: Point mutations: Y5V, N28T, K32Q, E45S, K65N, N78K, K97S, K103V, P108G, K134E, R145E, D156H, +160N

**Figure 31**

Oligonucleotide primers for site-directed mutagenesis of Der p 2

K6A	sense	OB43	42-mer	5' -CCGCTCGAGAAAAGAGATCAAGTCGATGTCGCCGATTGTGCC- 3'
	anti-sense	OB28	39-mer	5' -CGTTCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K15E	sense	OB44	67-mer	5' -CCGCTCGAGAAAAGAGATCAAGTCGATGTCAAAGATTGTGCC AACCATGAAATCAAAGAAGTTTGG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
H30N	sense	OB46	54-mer	5' -CGGGTACCAGGATGTCATGGTTCAGAACCATGTATCATTA CCGTGGTAAACC- 3'
	anti-sense	OB28	39-mer	5' -CGTTCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
E62S	sense	OB47	33-mer	5' -GCCTCAATCGATGGTTTATCAGTTGATGTTCCC- 3'
	anti-sense	OB48	33-mer	5' -GGGAACATCAACTGATAAACCATCGATTGAGGC- 3'
H74N	sense	OB49	32-mer	5' -CATGGCATGCAATTACATGAAATGCCCATTTGG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K82N	sense	OB50	50-mer	5' -CTACGCATGCCATTACATGAAATGCCCATTTGGTTAATGGACAA CAATATG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'

CCGCTCGAGAAAAGAGATCAAGTCGATGTCGCCGATTGTGCC- 3'



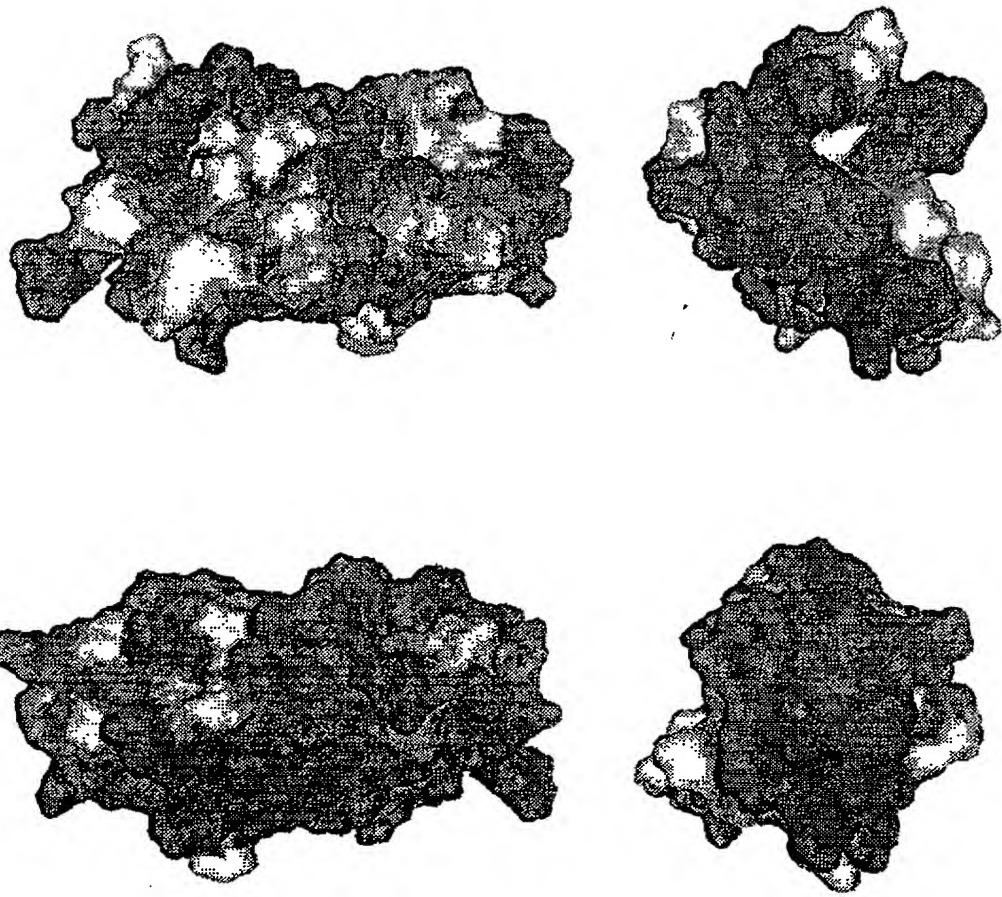


FIG. 33: Der p 2

10001245 114501

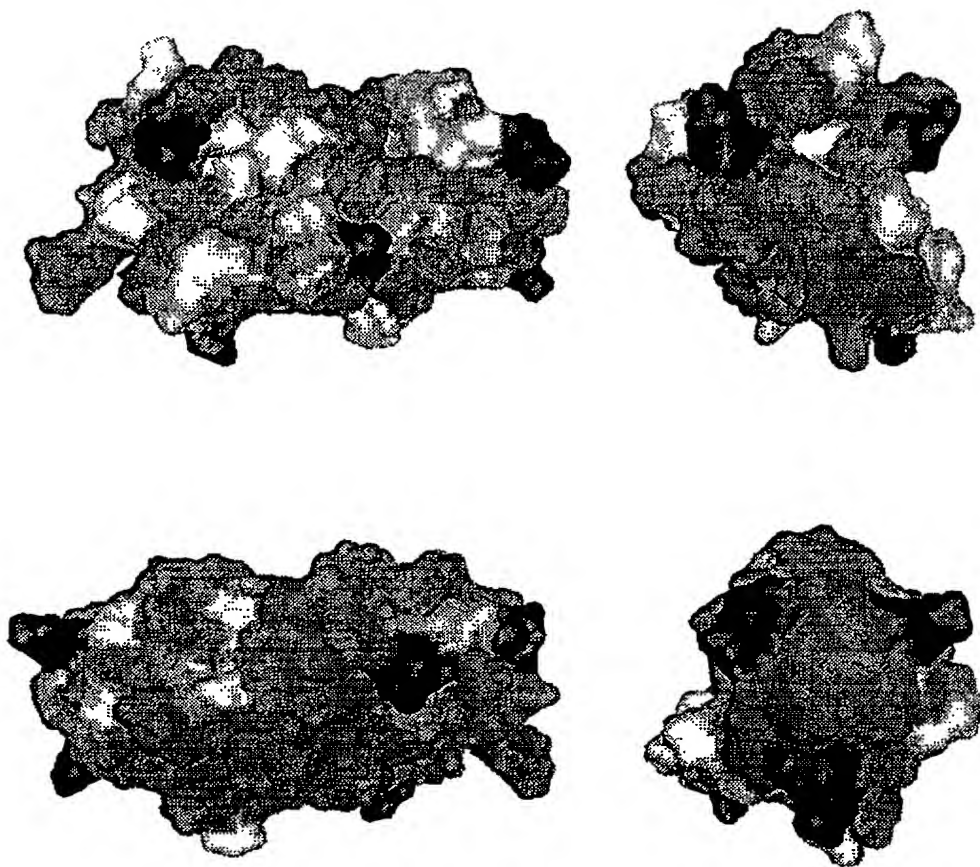
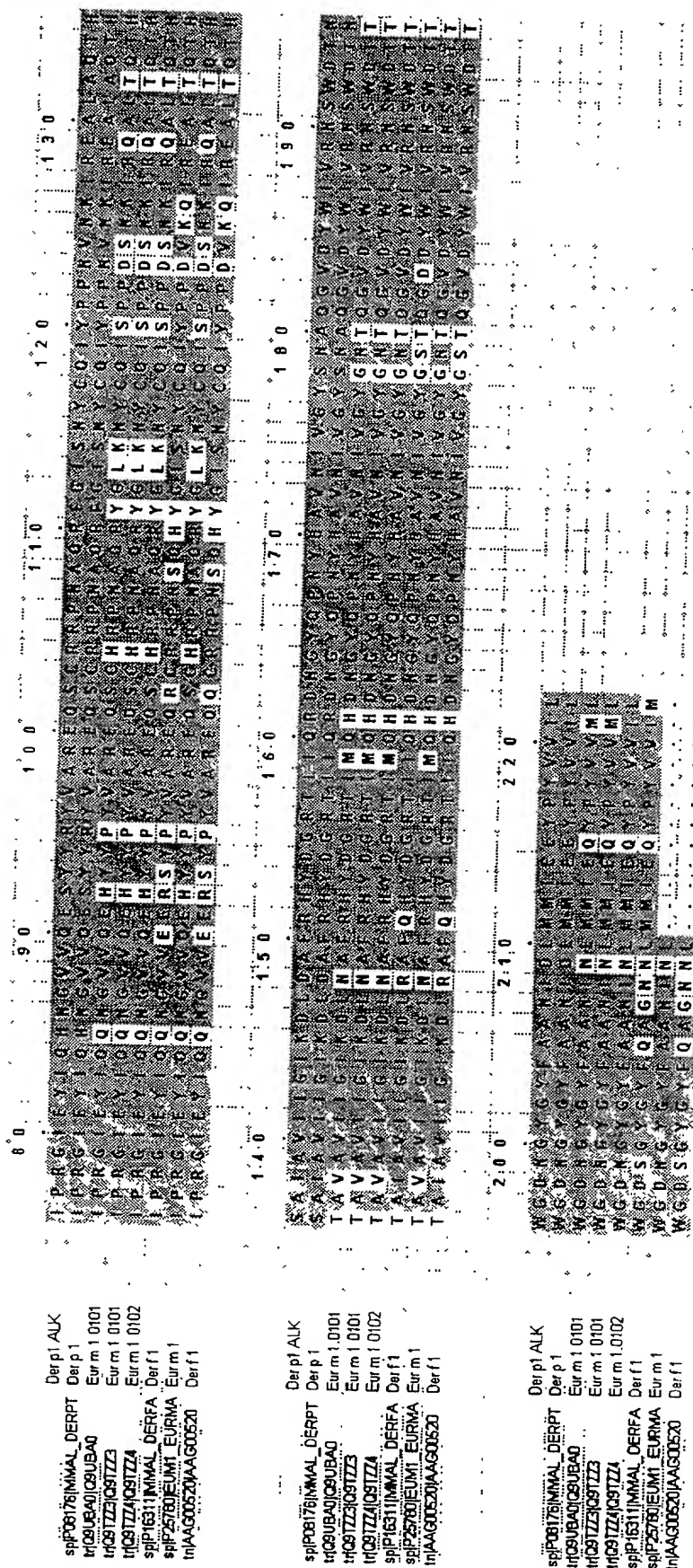


FIG. 34: Der p 2 mutant





Figure 35B (Der p 1)



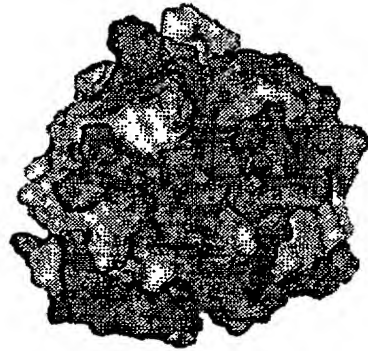
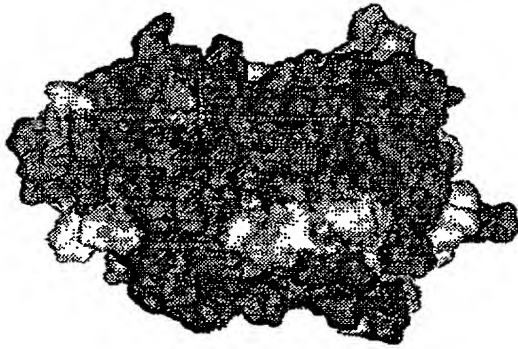
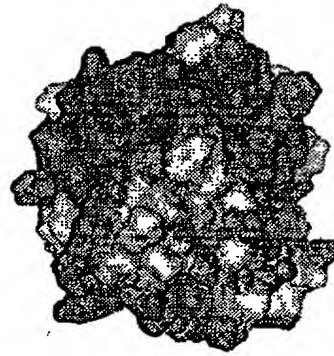
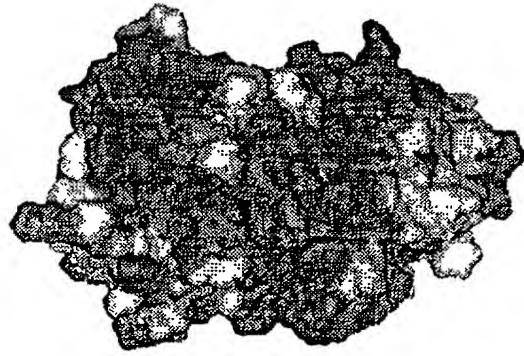


FIG. 36: Der p 1



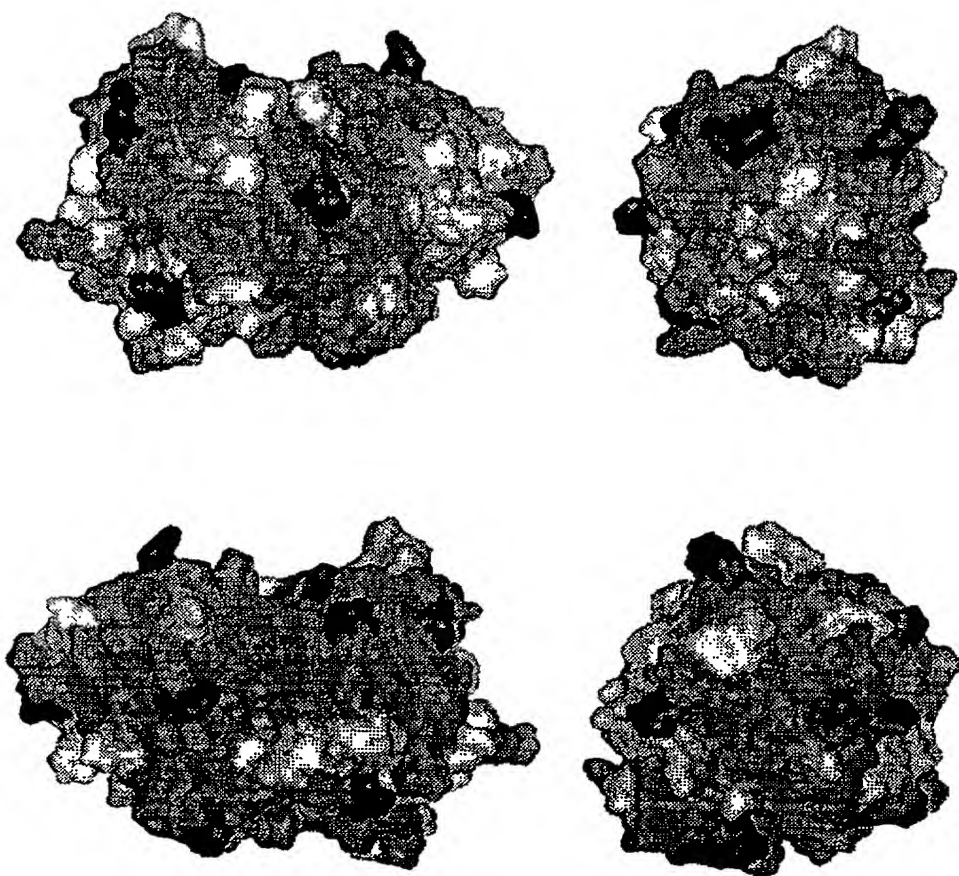


FIG. 37: Der p 1 mutant

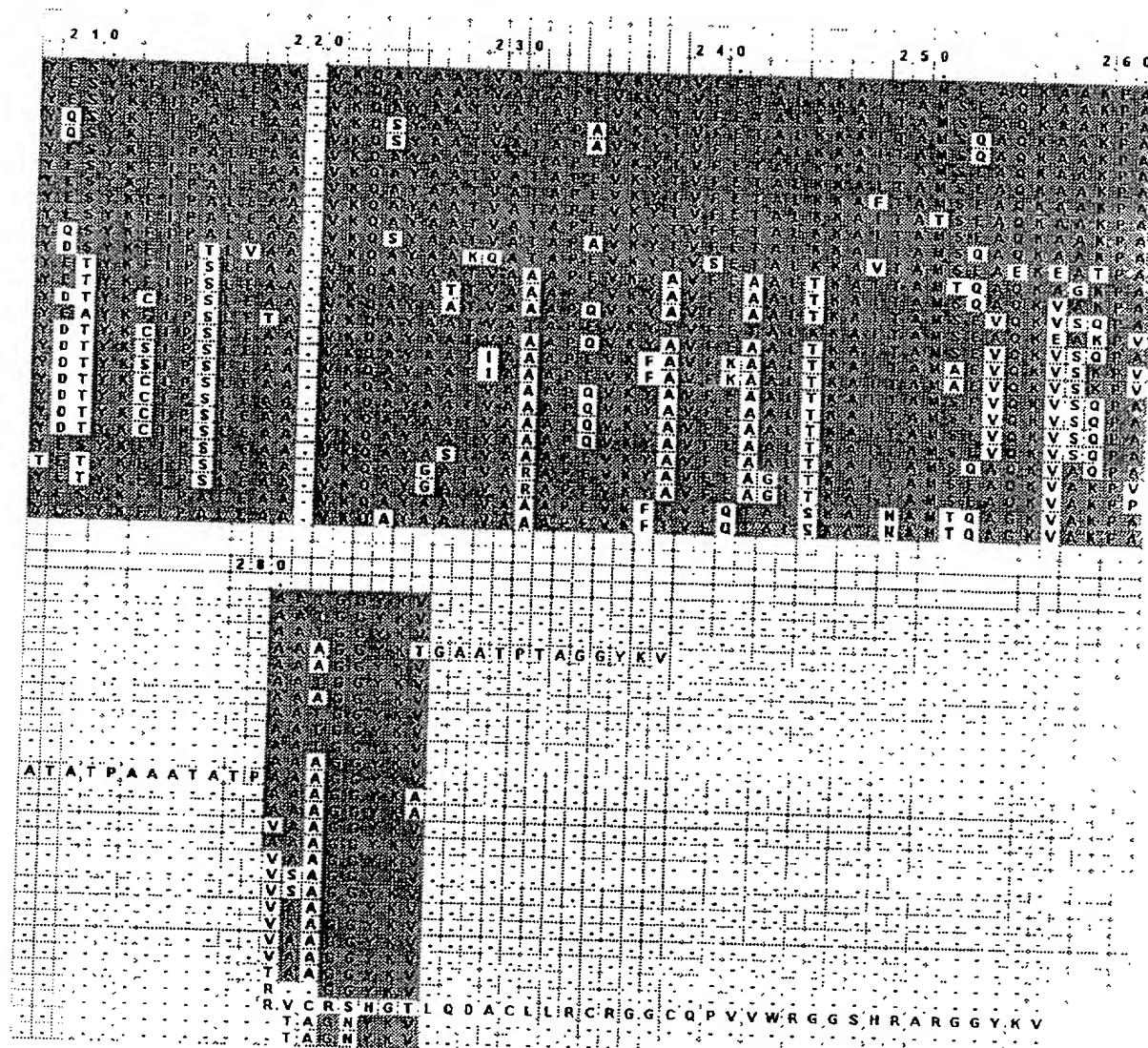
[illegible]

[illegible][illegible]





FIG. 38D (Phl p 5)



10001245 111501

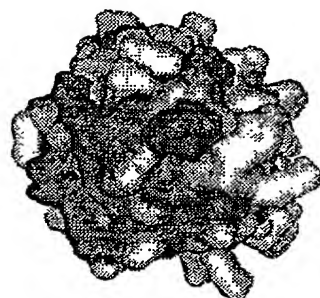
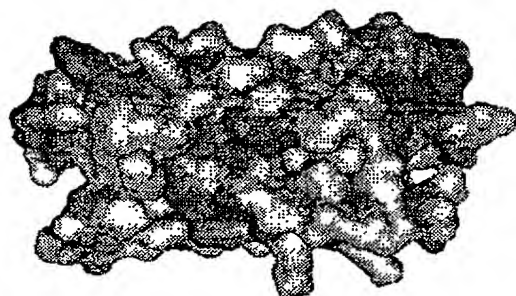
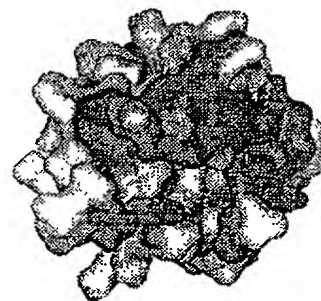
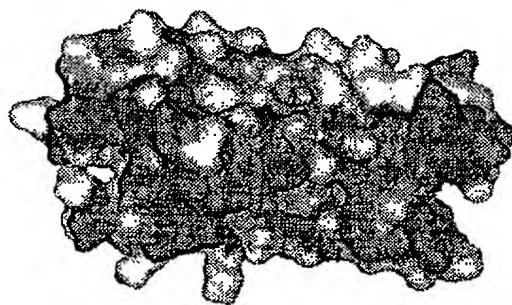


FIG. 39A: Phl p 5, Model A

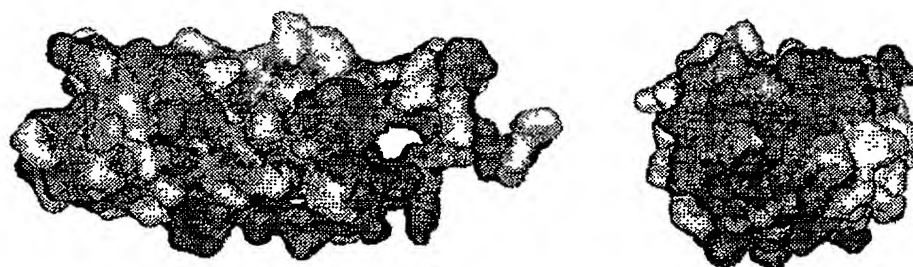
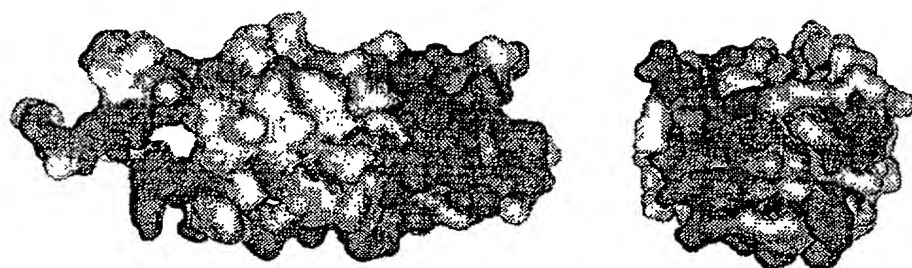


FIG. 39B: Phl p 5, Model B

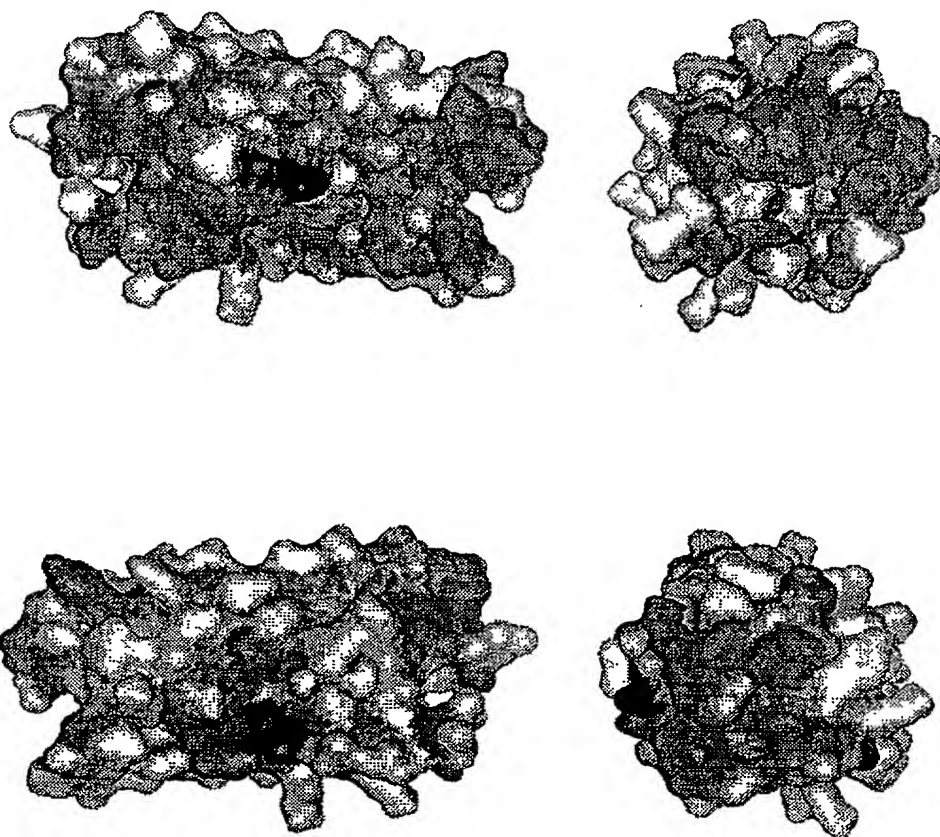


FIG. 40A: Phl p 5 mutant, Model A



40001245 44504

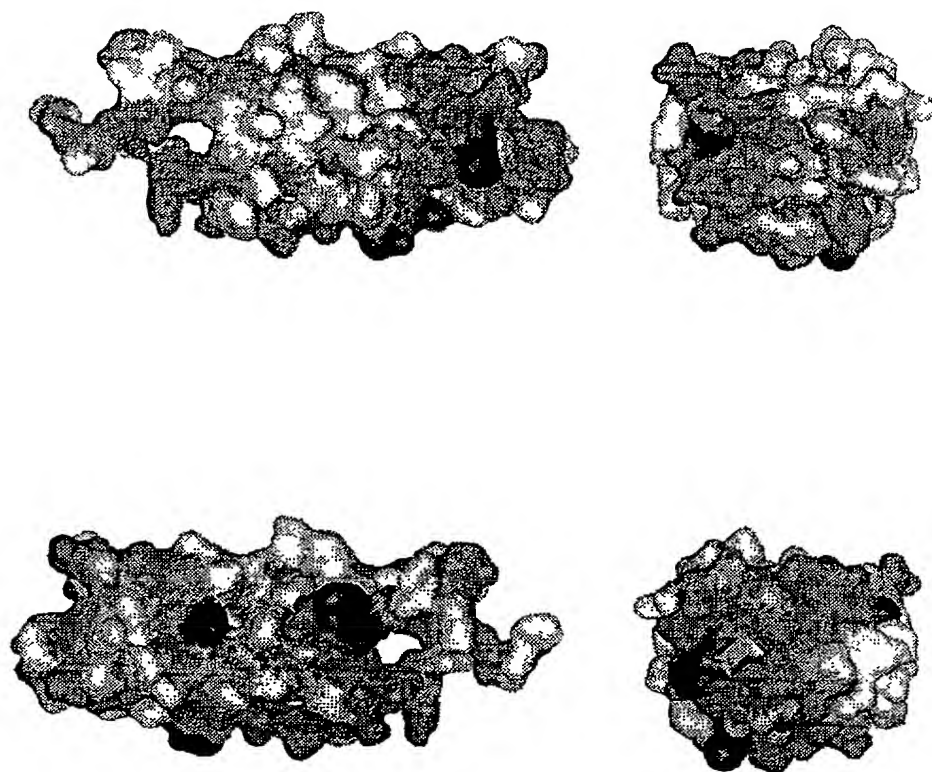


FIG. 40B: Phl p 5 mutant, Model B

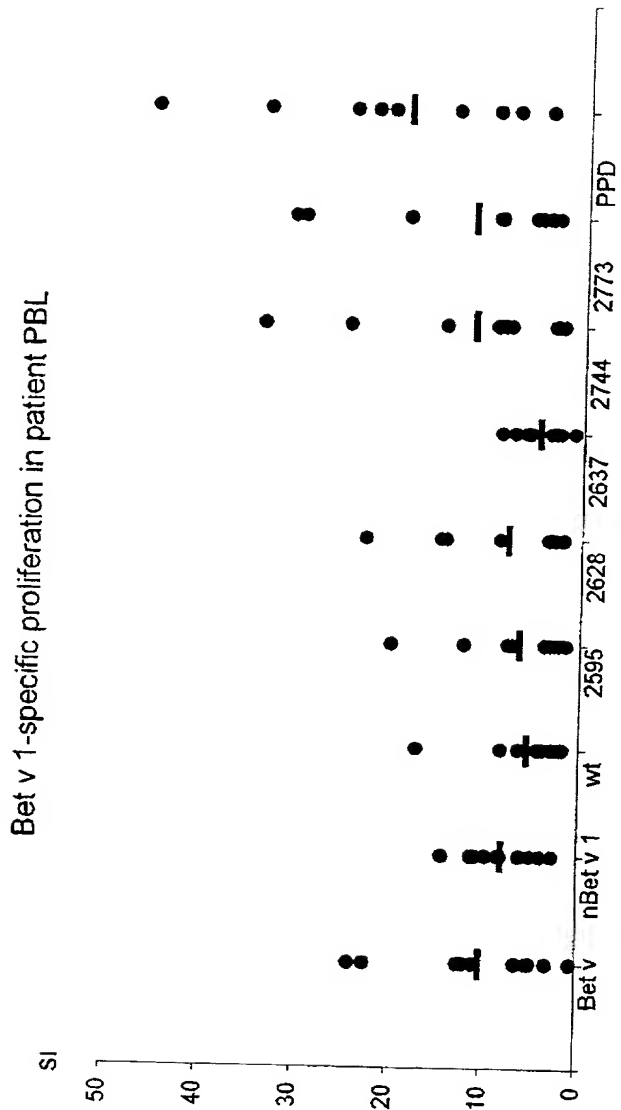


Figure 41: Stimulation of Bet v 1 samples

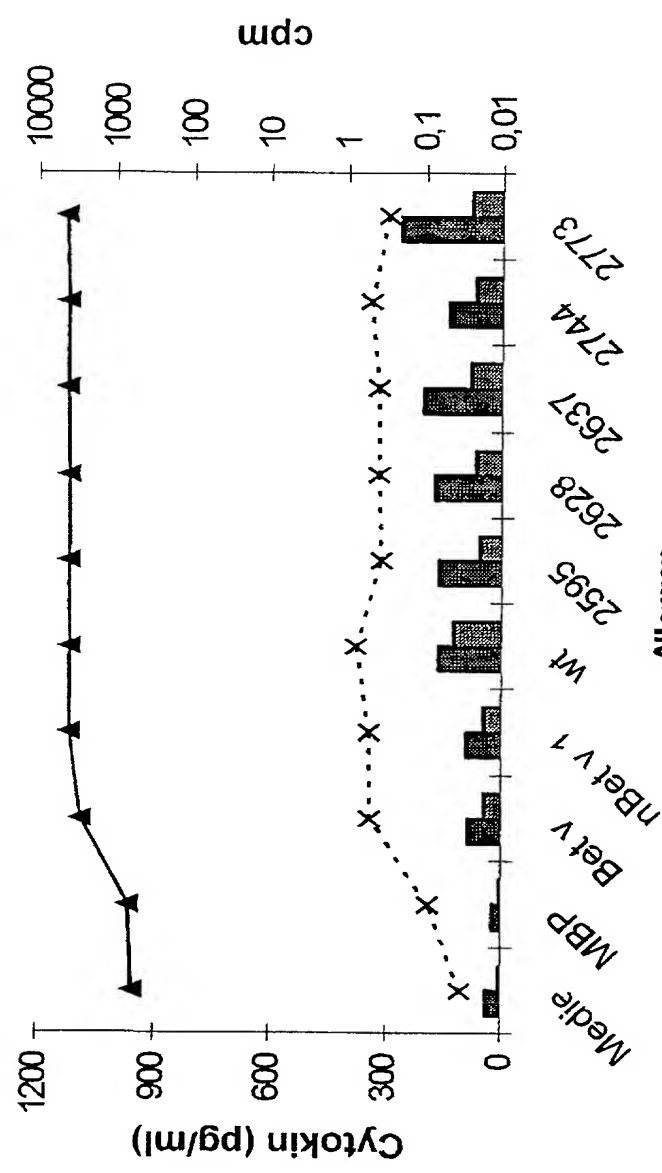
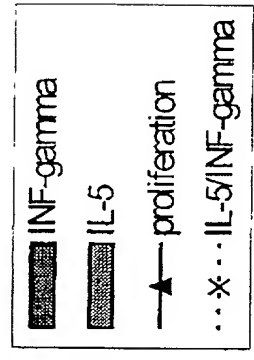


FIG. 42

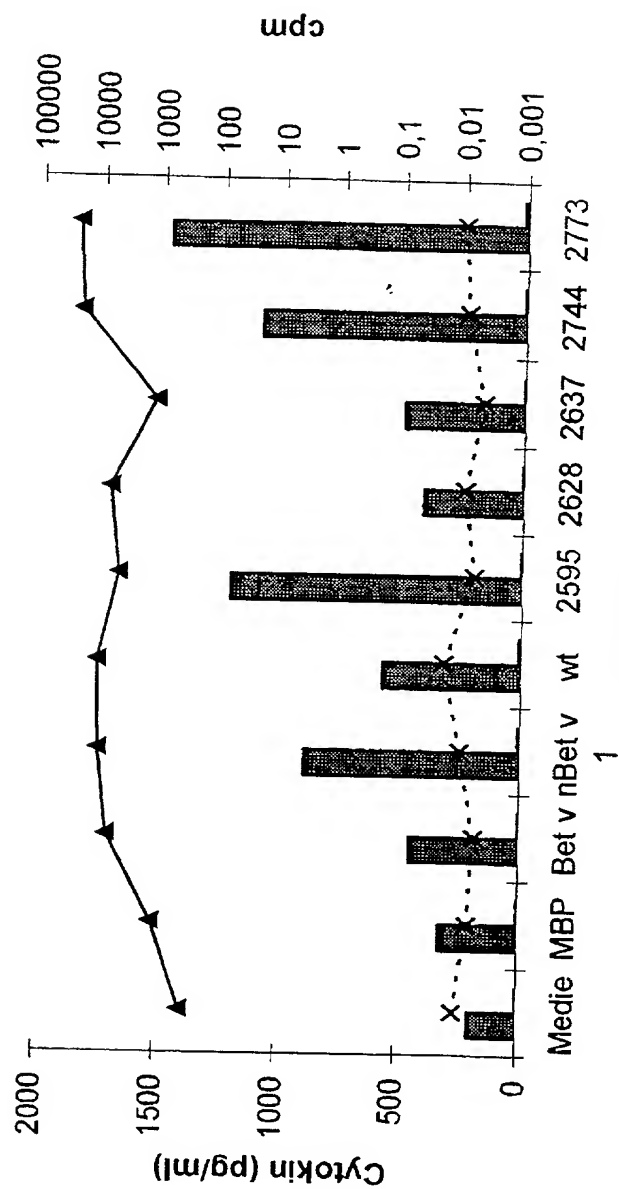
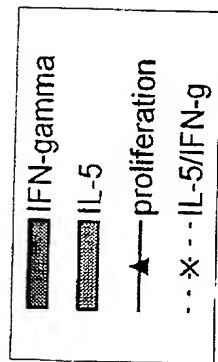
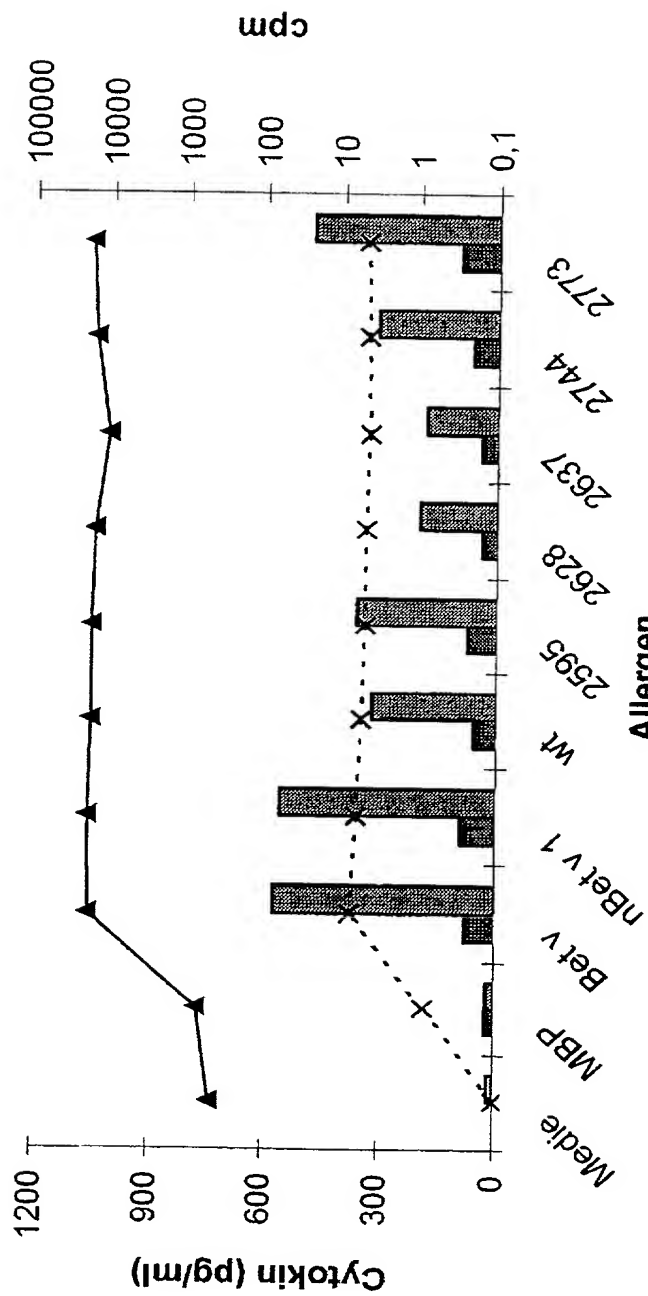
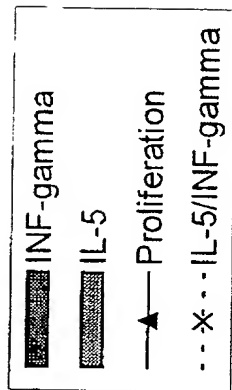


FIG. 43



Allergen

FIG. 44